Paroxetine Markedly Increases Plasma Concentrations of Ophthalmic Timolol; CYP2D6 Inhibitors May Increase the Risk of Cardiovascular Adverse Effects of 0.5% Timolol Eye Drops

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ABSTRACT

Although ophthalmic timolol is generally well tolerated, a significant fraction of topically administered timolol can be systemically absorbed. We investigated the effect of the strong CYP2D6 inhibitor paroxetine on the pharmacokinetics of timolol after ophthalmic administration. In a four-phase crossover study, 12 healthy volunteers ingested either paroxetine (20 mg) or placebo daily for 3 days. Administration of timolol and 1.49-fold (0.94–2.36) with 0.5% timolol, and that of timolol area under the plasma concentration–time curve (AUC) from time 0 to 12 hours was 1.61-fold (1.26–2.06-fold) and 1.78-fold (1.21–2.62), respectively. During paroxetine administration, six subjects on 0.5% timolol drops, but none on 0.1% timolol gel, had plasma timolol concentrations exceeding 0.7 ng/ml, which can cause systemic adverse effects in patients at risk. There was a positive correlation between the AUC from time 0 to 13 hours of paroxetine and the placebo phase AUC from time 0 to infinity of timolol after timolol 0.5% drops (P < 0.05), and a nonsignificant trend after timolol 0.1% gel, consistent with the role of CYP2D6 in the metabolism of both agents. In the orthostatic test, heart rate immediately after upright standing was significantly lower (P < 0.05) during the paroxetine phase than during the placebo phase at 1 and 3 hours after 0.5% timolol dosing. In conclusion, paroxetine and other CYP2D6 inhibitors can have a clinically important interaction with ophthalmic timolol, particularly when patients are using 0.5% timolol formulations.

Introduction

Glaucoma affects about 60 million people globally (Quigley, 2011). Timolol is a nonselective β-adrenoceptor blocking agent that is widely used as an ophthalmic preparation for the treatment of glaucoma (Brooks and Gillies, 1992). Although ophthalmic administration of timolol is generally well tolerated, even 80% of a topically administered timolol dose can be systemically absorbed (Shell, 1982; Korte et al., 2002). This can lead to systemic adverse effects affecting the cardiac, pulmonary, and central nervous systems (Van Buskirk, 1980; Van Buskirk and Fraunfelder, 1984).

Timolol is contraindicated in patients with sinus bradycardia, second- or third-degree atrio-ventricular block, overt cardiac failure, cardiogenic shock, cerebrovascular insufficiency, and asthma (Taniguchi and Kitazawa, 1997; Frishman et al., 2001). Systemically absorbed timolol may cause severe respiratory problems in patients who have bronchial hyper-reactivity or asthma. These adverse effects have been recognized for decades (Nelson et al., 1986). However, since ophthalmic timolol is applied topically, the systemic effects may be overlooked in clinical use. In fact, several case reports of severe systemic adverse effects of ophthalmic timolol have been published to alarm physicians of the risks (Minish and Herd, 2002; van der Velde et al., 2004; Carey, 2006; Müller et al., 2006; Calenda and Tourell, 2007; Patane’ et al., 2008; Schweitzer et al., 2008; Walia et al., 2011; Canpolat et al., 2013).

In glaucoma patients, there is a significant correlation between plasma concentrations of timolol and suppression of heart rate, particularly during exercise. Plasma timolol concentrations over 0.7 ng/ml are regularly associated with a heart rate reduction of 10 beats per minute or more at the maximal load in exercise tests (Nieminen et al., 2005b; Usitalo et al., 2006). To minimize systemic effects caused by timolol eye drops, their viscosity has been increased, so that the mean residence time of timolol on the ocular surface has increased. Accordingly, once-daily
doses of 0.1% timolol hydrogel decrease intraocular pressure about as effectively as twice-daily doses of 0.5% aqeous timolol (Rouland et al., 2002). However, the administration of 0.1% timolol hydrogel leads to lower plasma timolol concentration and cardiac toxicity compared with 0.5% aqueous timolol (Niño et al., 2002; Uusitalo et al., 2005, 2006).

Timolol is metabolized extensively to several metabolites, mainly by CYP2D6 (Lewis et al., 1985; Edeki et al., 1995; Nieminen et al., 2005a; Volotinen et al., 2007, 2010, 2011). Patients using ophthalmic timolol products are often elderly and may have several concomitant medications such as selective serotonin reuptake inhibitors, of which many are inhibitors of CYP2D6. Recently, the selective serotonin reuptake inhibitors paroxetine and fluoxetine were shown to be potent inhibitors of timolol metabolism in vitro (Volotinen et al., 2010, 2011), but their possible interaction with ophthalmic timolol in humans in vivo is not known.

Our aim was to investigate the effect of paroxetine on the plasma concentrations and pharmacokinetics of ophthalmic timolol in humans, and to investigate whether timolol formulation affects the extent of interaction. To achieve this, in a four-phase crossover study, 12 healthy volunteers were given a single dose of timolol 0.1% eye gel in phases 1 and 2 and a single dose of timolol 0.5% eye drops in phases 3 and 4 in both eyes on the third day of randomized pretreatment with oral placebo and paroxetine (20 mg once daily).

Materials and Methods

Subjects. Twelve healthy male volunteers of Caucasian origin (Table 1) participated in the study after giving written informed consent. The volunteers were ascertained to be healthy by medical history, physical examination, and laboratory tests before entering the study. None of the volunteers used continuous medication. The number of volunteers was estimated to be sufficient to detect a 30% change in the area under the plasma concentration–time curve (AUC) from time 0 to 12 hours (AUC0-12h) of timolol with a power of 80% (α-level 5%).

Study Design. The study protocol was approved by the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District and by the National Agency for Medicines, and the study was reported at clinicaltrials.gov (trial identifier: NCT00879009) before its start. This was a placebo-controlled (placebo versus paroxetine), crossover study with four phases (Fig. 1). All 12 participants received 0.1% timolol eye gel in study phases 1 and 2 following the randomized placebo and paroxetine pretreatments, and then 0.5% timolol eye drops in phases 3 and 4 following the randomized placebo and paroxetine pretreatments. The phases were separated by washout periods of at least 4 weeks.

Paroxetine 20 mg (Seroxat, 20-mg tablets; GlaxoSmithKline Oy, Espoo, Finland) or placebo was administered once daily at 8 a.m. for 3 days during all four phases. The third pretreatment dose of each phase was ingested after an overnight fast. To ensure blinding, paroxetine tablets and placebo were encapsulated in colored gelatin capsules and filled with microcrystalline cellulose.

On the third pretreatment days, 1 hour after the last pretreatment dose, each subject was given one drop of either of the ophthalmic timolol products into the lower conjunctival sac of both eyes in supine position. The subjects stayed in the supine position for 3 minutes after instillation, keeping eyes lightly closed. In phases 1 and 2, the timolol product was Timosan 0.1% eye gel (Santen Oy, Tampere, Finland), and in phases 3 and 4, the product was Oftan Timolol 0.5% eye drops (Santen Oy). These formulations differ in their viscosity. The 0.1% timolol hydrogel is more viscous than the 0.5% aqueous timolol. Each subject received all four treatment combinations.

Food intake was identical in all four phases. On the study days, breakfast was ingested about 30 minutes after intake of the pretreatment drugs. A standardized warm meal was served after 3 hours, and snacks were given after 7 and 11 hours after timolol. The use of coffee or any caffeine-containing drinks was not permitted until the 7-hour time point.

Sampling. On the days of timolol administration, timed blood samples were drawn from a cannulated forearm vein just before the ingestion of the last pretreatment dose, and at 20, 40, 60, and 90 minutes and 2, 3, 4, 5, 6, 7, 9, and 12 hours after the administration of timolol. Blood samples (10 ml each) were taken into EDTA-containing tubes. Plasma was separated by centrifugation within 30 minutes and stored in triplicate polypropylene tubes at −70°C until analysis.

Determination of Drug Concentrations. Plasma samples (1.0 ml) spiked with 200 μl of formic acid (30%) and 100 μl of internal standard (timolol-d3 25 ng/ml in 50% methanol) were vortex mixed and centrifuged for 5 minutes at 2000g. The samples were applied to the preconditioned mixed-mode cation exchange extraction cartridges (Waters, Milford, MA), and the samples were allowed to pass through the cartridges. The cartridges were then washed with 1 ml of 0.1 M hydrochloric acid followed by 1 ml of methanol. Finally, the analytes were eluted twice with 0.6 ml of 2% ammonium hydroxide in methanol. The eluent was evaporated to dryness (50°C) under a nitrogen gas stream, and the residues were dissolved in 70 μl of acetonitrile-water (10:90 v/v) and transferred into autosampler vials. Concentrations of timolol and two of its metabolites (M6,

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Weight</th>
<th>BMI</th>
<th>CYP2D6</th>
<th>Timolol 0.1% phase</th>
<th>Timolol 0.5% phase</th>
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<tr>
<td></td>
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<td></td>
<td>Paroxetine AUC0-13 h</td>
<td>Timolol Cmax During Paroxetine</td>
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<td></td>
<td></td>
<td>ng/ml</td>
<td>ng/ml</td>
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<tr>
<td>Years</td>
<td>kg</td>
<td>kg/m²</td>
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<td></td>
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<td></td>
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<tr>
<td>1</td>
<td>24</td>
<td>86</td>
<td>26.0</td>
<td>UM</td>
<td>29.2</td>
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</tr>
<tr>
<td>2</td>
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<td>21.0</td>
<td>EM</td>
<td>72.1</td>
<td>0.056</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>87</td>
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</tr>
<tr>
<td>4</td>
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<tr>
<td>5</td>
<td>22</td>
<td>66</td>
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<td>EM</td>
<td>117.6</td>
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<tr>
<td>6</td>
<td>21</td>
<td>68</td>
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<td>7</td>
<td>24</td>
<td>74</td>
<td>24.0</td>
<td>EM</td>
<td>115.5</td>
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<tr>
<td>8</td>
<td>22</td>
<td>75</td>
<td>24.5</td>
<td>EM</td>
<td>97.9</td>
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</tr>
<tr>
<td>9</td>
<td>23</td>
<td>80</td>
<td>22.5</td>
<td>EM</td>
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<tr>
<td>10</td>
<td>23</td>
<td>84</td>
<td>22.5</td>
<td>EM</td>
<td>34.7</td>
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<tr>
<td>11</td>
<td>21</td>
<td>65</td>
<td>19.5</td>
<td>EM</td>
<td>61.6</td>
<td>0.100</td>
</tr>
<tr>
<td>12</td>
<td>24</td>
<td>76</td>
<td>23.3</td>
<td>EM</td>
<td>156.9</td>
<td>0.273</td>
</tr>
</tbody>
</table>

Average $± 2$ 78 $± 10$ 23.0 $± 2.1$ 95.5 (82%) 0.11 (57%) 1.54 (0.94–2.6) 78.8 (79%) 0.64 (72%) 1.79 (0.41–4.0)

BMI: body mass index.
lactic acid formation to morpholine ring, and M7, dihydroxylation to morpholine ring; Supplemental Fig. 1) were measured using an API 3000 liquid chromatography–tandem mass spectrometry system (Sciex Division of MDS, Toronto, Ontario, Canada). Chromatography was performed on an Atlantis T3 column (2.1 × 100 mm; Waters) using gradient elution. The mobile phase consisted of 0.01% formic acid and acetonitrile. Timolol-d8 served as an internal standard. The mass spectrometer was operated in the positive multireaction monitoring detection mode with electrospray ionization. The ion transitions monitored were the sum of m/z 317 to m/z 261 and m/z 317 to m/z 74 for timolol, m/z 349 to m/z 293 for M6 and M7, and m/z 322 to m/z 266 for timolol-d8. The metabolites were monitored using the most sensitive ion transition, m/z 349 to m/z 293, for both M6 and M7. Prior to analysis, M6 and M7 were separated by liquid chromatography mass spectrometry and identified according to the known characteristic differences in metabolite fragmentation patterns, m/z 176 for M6 and m/z 144 for M7 (Volotinen et al., 2010). The lower limit of quantification for timolol was 0.01 ng/ml, and interday coefficients of variation (CVs) were 9.5% at 0.01 ng/ml, 3.9% at 0.2 ng/ml, and 4.8% at 1.0 ng/ml (n = 6). Because authentic metabolite standards for M6 and M7 were not available, their concentrations are given in arbitrary units per milliliter relative to the ratio of their peak height to that of the internal standard in the chromatogram. These ratios were shown to be linear in relevant concentrations using the plasma dilution technique. The limit of quantification for M6 and M7 was based on a signal-to-noise ratio of more than 10:1. The plasma concentrations of paroxetine were determined using the Applied Biosystems API 2000 Q Trap liquid chromatography–tandem mass spectrometry system (Sciex Division of MDS), as described earlier (Chu and Metcalfe, 2007). Citalopram served as the internal standard. The limit of quantification for paroxetine was 0.3 ng/ml, and interday CVs were 1.5–8.5% at relevant plasma concentrations.

**Pharmacokinetics.** The pharmacokinetics of timolol were characterized by peak concentration (Cmax), time to Cmax (tmax), areas under the plasma concentration–time curve [AUC0–12h and AUC from time 0 to infinity (AUC0–∞)], and half-life (t1/2), calculated by noncompartmental analysis using MK-Model version 5.0 (Biosoft, Cambridge, UK). The t1/2 was calculated by the equation t1/2 = ln2/klo. For timolol metabolites M6 and M7, only Cmax, tmax, and AUC0–12h values, and the ratios of their AUC0–12h to the AUC0–12h of timolol, are given. The AUC values were calculated using the linear trapezoidal rule for the rising phase of the plasma concentration–time curve and the log-linear trapezoidal rule for the descending phase, with extrapolation to infinity, when appropriate, by dividing the last measured concentration by klo. The pharmacokinetics of paroxetine were characterized by its AUC from time 0 to 13 hours (AUC0–13h), calculated from its last administration, i.e., 1 hour before the application of ophthalmic timolol.

**Pharmacodynamics.** Systolic and diastolic blood pressures and heart rate were recorded from the forearm with an automatic oscillometric blood pressure monitor (Omron M5-1; Omron Healthcare Europe BV, Hoofddorp, The Netherlands), with the participant in a sitting position, after each blood sampling. The primary pharmacodynamic variable was heart rate in the orthostatic test, which was carried out before timolol application and at 1 and 3 hours after timolol application. In this test, systolic and diastolic blood pressures and heart rate were recorded in a supine position, immediately after upright standing, and after 2-minute standing. The parameters were measured in duplicate and the mean value was taken. An electrocardiogram was recorded before and 2 hours after timolol application for safety purposes.

**Genotyping for CYP2D6.** The participants were genotyped for the CYP2D6*3, *4, *6, *9, *10, and *41 alleles, and whole-gene deletion (*5) and duplication using a two-step multiplex primer extension method (Sistonen et al., 2005). Individuals were defined as poor metabolizers (PMs), intermediate metabolizers, extensive metabolizers (EMs), and ultra-rapid metabolizers (UMs) according to conventional criteria (Zanger et al., 2004).

**Statistical Analysis.** The results are expressed as mean values ± S.D. or as geometric means and CVs (90% confidence intervals) in the text, tables, and figures, unless otherwise indicated. The pharmacokinetic and pharmacodynamic variables between the placebo and paroxetine phases were compared by paired-samples t test or repeated-measures analysis of variance with treatment and treatment sequence as factors, as appropriate. Logarithmic transformation was used for pharmacokinetic variables, except for tmax. For log-transformed variables, geometric means and CVs are given, the CV representing a measure of percentage variability (standard deviation in relation to mean) in observed values. The differences were considered statistically significant at P < 0.05, and no adjustment was made for multiple testing. The tmax data were compared using the Wilcoxon signed-rank test. The Pearson correlation coefficient and linear regression analysis were used for investigation of relationships between the pharmacokinetic variables and the extent of the interaction of paroxetine with timolol formulations.

**Results**

**Plasma Concentrations and Pharmacokinetics of Timolol.** Plasma timolol profiles were affected by both the formulation and the paroxetine pretreatment (Fig. 2). During the placebo phase, the geometric mean values of timolol Cmax, AUC0–12h, and AUC0–∞ were 6.1, 6.7, and 4.5 times higher, respectively, with 0.5% aqueous timolol than with 0.1% timolol gel (Table 2). The plasma t1/2 of timolol with the 0.1% gel was 10.5 hours, i.e., significantly (P < 0.001) longer than the t1/2 of 4.8 hours observed with the 0.5% aqueous timolol drops.

During the paroxetine phase, the geometric means of timolol Cmax and AUC0–12h with 0.1% timolol gel were 1.53 and 1.61 times higher, respectively, than during the placebo phase (P < 0.01), and those with
0.5% timolol drops were 1.49 and 1.78 times higher than during the placebo phase, respectively (Table 2). Paroxetine pretreatment did not change the plasma half-life of timolol with either of the formulations (Fig. 2; Table 2).

After the application of 0.1% timolol gel, plasma timolol concentrations remained below 0.4 ng/ml in all 12 subjects, in both the placebo and paroxetine phases (Table 1). With the 0.5% aqueous timolol drops, plasma timolol concentrations exceeded 0.7 ng/ml in two subjects during the placebo phase, and in six subjects during the paroxetine phase.

Paroxetine reduced the $C_{\text{max}}$ and $\text{AUC}_{0-12\text{h}}$ values of the timolol metabolite M7 with both timolol formulations ($P < 0.05$), but had no significant effect on those of M6 (Fig. 2; Table 2). However, paroxetine significantly decreased both the $\text{M6/timolol AUC}_{0-12\text{h}}$ ratio (by about 50%) and the $\text{M7/timolol AUC}_{0-12\text{h}}$ ratio (by about 65%) (Table 2).
CYP2D6 Genotypes and Variability in the Effect on Timolol.
Ten of the participants had an EM CYP2D6 metabolizer genotype, one participant had a PM genotype, and one had a UM genotype (Table 1). The effect of paroxetine on timolol AUC values was roughly similar in the UM subject (no. 1) compared to that in the EM participants. In the PM subject (no. 3), paroxetine did not increase timolol AUC values despite very high plasma paroxetine concentrations (Fig. 3, C and E).

Paroxetine versus Timolol Exposures. There was only little intrainterventional variation in paroxetine AUC0-12h values between the two paroxetine phases, despite over 10-fold interindividual variation (Table 1), resulting in a good correlation between the two paroxetine phases (Fig. 3A). During both phases, paroxetine AUC0-12h was about 5 times greater in the CYP2D6 PM subject (no. 3) than in the EM participants, and more than 10 times greater than in the UM participant (no. 1).

There was a significant positive correlation between the AUC0-12h of paroxetine and the placebo-phase AUC0-12h of timolol after timolol 0.5% drops (P < 0.05), and a nonsignificant trend after timolol 0.1% gel (Fig. 3, B and D). Although the subject with the PM genotype had a very large AUC0-12h of timolol after both formulations during the placebo phase, the UM subject did not differ clearly from the EM participants.

There was no positive correlation between paroxetine exposure and its effect on timolol. In fact, there was a significant inverse correlation (r = −0.62, P < 0.05) between the paroxetine AUC0-13h and the fold increase of timolol AUC0-12h by paroxetine after the application of 0.5% timolol eye drops (Fig. 3C). Although there was no apparent difference between the formulations in the average extent of interaction, the AUC of timolol increased more than 2-fold in seven of the 10 EM subjects with the 0.5% formulation and in only one subject with the 0.1% formulation (Fig. 3, C and E).

Effects on Heart Rate and Blood Pressure. Heart rate decreased from baseline until 3 hours (lunch) after timolol application during all four study phases (P < 0.001; Supplemental Fig. 2). After 0.5% timolol eye drops, the mean decrease of heart rate exceeded 10 beats per minute at 3 hours postdose during the paroxetine phase only, and the average heart rate 0–12 hours postdose was lower (P < 0.05) during the paroxetine phase than during the placebo phase (Supplemental Table 1). The average blood pressures were slightly higher during the paroxetine phases than during the placebo phases (P < 0.05), and a similar difference was observed already at baseline.

In the orthostatic test, heart rate immediately after upright standing was significantly lower (P < 0.05) during the paroxetine phase than during the placebo phase at 1 and 3 hours after 0.5% timolol dosing (Fig. 4; Supplemental Figs. 3 and 4). However, no such difference was observed before timolol dosing or after 0.1% timolol dosing.

### Discussion
To our knowledge, this is the first controlled study in which a CYP2D6 inhibitor has been shown to increase the systemic plasma concentrations of a drug (timolol) applied topically on the eyes. Our results are in line with an earlier in vitro study which showed that paroxetine strongly inhibits the formation of all detected timolol metabolites, with IC50 values between 0.5 and 2.5 μM (Volotinen et al., 2010).

Paroxetine 20 mg daily has increased the AUCs of desipramine and metoprolol about 5-fold in CYP2D6 EMs (Brøsen et al., 1993; Hemeryck et al., 2000), suggesting that the 20 mg/day dosage of paroxetine reduces CYP2D6 activity by at least 80%. Furthermore, a single 20-mg dose of paroxetine increases the metabolic ratio of (+)-tramadol >4-fold, consistent with almost 80% inhibition of CYP2D6 (Nielsen et al., 2010). However, it should be noted that, as paroxetine is a mechanism-based inhibitor of CYP2D6, which can reduce its own clearance due to a time-dependent autoinhibitory effect (Bertelsen et al., 2003; Sawamura et al., 2004), its CYP2D6-inhibiting effect may increase further during prolonged use. Thus, our short treatment may underestimate the extent of interaction, particularly in patients with ultra-rapid CYP2D6 genotype or reduced kidney function. Furthermore, the CYP2D6 inhibitory effect of paroxetine is dose-dependent (Sawamura et al., 2004; Nielsen et al., 2010). Thus, the interaction of

### Table 2
Pharmacokinetic variables of timolol after administration of a single dose of 0.1% eye gel or 0.5% eye drops to both eyes after oral pretreatment with placebo or 20 mg of paroxetine daily for 3 days in 12 healthy male volunteers. Data are given as the geometric mean with geometric variation coefficients (GeoCV%) and geometric mean ratios with 95% confidence interval (95% CI); tmax values are given as the median and range. Geometric CV% values give a measure of percentage variability (standard deviation in relation to mean) in observed values.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo (Control)</th>
<th>Paroxetine (GeoCV%)</th>
<th>Geometric Mean Ratio (95% CI)</th>
<th>p</th>
<th>Placebo (Control)</th>
<th>Paroxetine (GeoCV%)</th>
<th>Geometric Mean Ratio (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Timolol</strong></td>
<td></td>
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<tr>
<td>Cmax (ng/ml)</td>
<td>0.071 (67)</td>
<td>0.11 (57)</td>
<td>1.53 (1.23–1.91)</td>
<td>0.002</td>
<td>0.43 (70)</td>
<td>0.64 (72)</td>
<td>1.49 (0.94–2.36)</td>
<td>0.083</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>0.33 (0.33–0.67)</td>
<td>0.33 (0.33–0.67)</td>
<td></td>
<td></td>
<td>0.5 (0.33–4)</td>
<td>1.25 (0.33–4)</td>
<td></td>
<td>0.362</td>
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<tr>
<td>t1/2 (h)</td>
<td>10.5 (50)</td>
<td>10.6 (62)</td>
<td>1.00 (0.80–1.27)</td>
<td>0.964</td>
<td>4.8 (49)</td>
<td>5.0 (44)</td>
<td>1.05 (0.86–1.27)</td>
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<tr>
<td>AUC0-12h (ng-h/ml)</td>
<td>0.340 (48)</td>
<td>0.547 (35)</td>
<td>1.61 (1.26–2.06)</td>
<td>0.002</td>
<td>2.28 (68)</td>
<td>4.06 (48)</td>
<td>1.78 (1.21–2.62)</td>
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<td>AUC0-12h (ng-h/ml)</td>
<td>0.635 (52)</td>
<td>1.05 (37)</td>
<td>1.65 (1.37–1.98)</td>
<td>0.0001</td>
<td>2.90 (55)</td>
<td>5.25 (39)</td>
<td>1.81 (1.32–2.48)</td>
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<tr>
<td>Cmax (au/ml)</td>
<td>0.37 (62)</td>
<td>0.29 (35)</td>
<td>0.79 (0.57–1.09)</td>
<td>0.139</td>
<td>2.1 (67)</td>
<td>1.8 (67)</td>
<td>0.86 (0.55–1.36)</td>
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<tr>
<td>tmax (h)</td>
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<td>4.5 (2–7)</td>
<td>0.312</td>
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<td>4 (2–6)</td>
<td>5 (3–9)</td>
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<tr>
<td>AUC0-12h (au-h/ml)</td>
<td>2.51 (70)</td>
<td>1.90 (62)</td>
<td>0.76 (0.51–1.14)</td>
<td>0.158</td>
<td>15.7 (62)</td>
<td>14.7 (61)</td>
<td>0.94 (0.63–1.40)</td>
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<td>M6/timolol AUC0-12h - ratio (au/ng)</td>
<td>7.4 (40)</td>
<td>3.5 (45)</td>
<td>0.47 (0.34–0.65)</td>
<td>0.0004</td>
<td>6.9 (40)</td>
<td>3.6 (34)</td>
<td>0.53 (0.42–0.67)</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>M7-metabolite</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (au/ml)</td>
<td>0.60 (101)</td>
<td>0.32 (45)</td>
<td>0.53 (0.38–0.74)</td>
<td>0.002</td>
<td>3.6 (83)</td>
<td>2.0 (65)</td>
<td>0.56 (0.35–0.89)</td>
<td>0.02</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>4.5 (2–7)</td>
<td>3.5 (1–5)</td>
<td>0.058</td>
<td></td>
<td>4 (2–5)</td>
<td>4.5 (2–9)</td>
<td>0.147</td>
<td></td>
</tr>
<tr>
<td>AUC0-12h (au-h/ml)</td>
<td>4.66 (112)</td>
<td>2.48 (56)</td>
<td>0.53 (0.39–0.73)</td>
<td>0.001</td>
<td>25.5 (62)</td>
<td>15.8 (62)</td>
<td>0.62 (0.40–0.95)</td>
<td>0.031</td>
</tr>
<tr>
<td>M7/timolol AUC0-12h - ratio (au/ng)</td>
<td>13.7 (69)</td>
<td>4.5 (38)</td>
<td>0.33 (0.25–0.43)</td>
<td>0.000003</td>
<td>11.2 (75)</td>
<td>3.9 (36)</td>
<td>0.35 (0.26–0.47)</td>
<td>0.00001</td>
</tr>
</tbody>
</table>

au, arbitrary unit.
paroxetine with ocular timolol products may be particularly strong in patients using the highest recommended doses of paroxetine, 50–60 mg daily.

Since paroxetine strongly inhibits CYP2D6 activity, and it has been estimated that CYP2D6 is responsible for >90% of the formation of the main primary metabolites of timolol (Volotinen et al., 2007), the interaction caused by paroxetine was smaller than expected, even when considering that almost 20% of the oral timolol dose is excreted unchanged by the kidneys (Wasson et al., 1980; Fourtillan et al., 1981). However, it should be recognized that timolol undergoes first-pass metabolism, and only 50–70% of oral timolol is bioavailable (Wilson et al., 1982). Thus, about one-third of the bioavailable fraction of timolol is excreted unchanged in urine. In the current study, the first-pass metabolism of timolol was partially avoided by ophthalmic

Fig. 3. Relationship of the AUC_{0-13h} of paroxetine during the timolol 0.1% hydrogel phase (A), the total AUC of timolol after timolol 0.5% drops in the placebo phase (B), and the fold increase in the total AUC of timolol after timolol 0.5% drops caused by paroxetine (C) to the AUC_{0-13h} of paroxetine during the timolol 0.5% drop phase. Relationship of the total AUC of timolol after timolol 0.1% hydrogel in the placebo phase (D) and the fold increase in the total AUC of timolol after timolol 0.1% hydrogel caused by paroxetine (E) to the AUC_{0-13h} of paroxetine during the timolol 0.1% hydrogel phase.
administration. Consequently, renal elimination may have approached one-third of timolol clearance, limiting the role of CYP2D6 to $<70\%$ of timolol clearance. This probably explains why the increase in timolol AUC caused by paroxetine was not greater than 3.4-fold in any of the subjects.

Our results are in line with findings showing that the strong CYP2D6 inhibitor quinidine increased plasma concentrations of timolol (Edeki et al., 1995) when timolol was administered to the nasal mucosa, instead of dosing timolol to the eye according to clinical practice. The administration of quinidine together with timolol drops also resulted in a reduction in exercise-induced heart rate, when compared with 0.5% timolol alone. In another study, administration of cimetidine, a nonselective inhibitor of several cytochrome P450 enzymes (Martinez et al., 1999), with ophthalmic aqueous 0.5% timolol resulted in reductions in resting heart rate and intraocular pressure in healthy volunteers (Ishii et al., 2000).

The AUC of orally administered timolol has been 2–4 times larger in CYP2D6 PM subjects compared with EMs (Alvan et al., 1982; Lewis et al., 1985; McGourty et al., 1985). In the present study, the only PM subject had an exposure to paroxetine about 5 times higher than the average exposure to paroxetine, and the subject’s systemic timolol exposure during the placebo phase was about 2-fold, compared with that in EMs. Furthermore, paroxetine caused no appreciable further increase in the subject’s timolol exposure. There was an apparently paradoxical inverse relationship between the plasma AUC$_{0\text{;}12\text{;h}}$ of paroxetine and the fold increase of timolol AUC$_{0\text{;}12\text{;h}}$ by paroxetine (Fig. 4, C and E). Self-evidently, the paroxetine-timolol interaction is minor or totally lacking in subjects with weakly functional or nonfunctional CYP2D6, despite their high paroxetine concentrations.

As expected, the plasma concentrations of paroxetine were lower in the UM subject than in EMs during both paroxetine phases. However, the subject’s average timolol concentrations were not particularly low after 0.5% timolol during the placebo phase. The higher-than-expected timolol concentrations in this subject might be explained by individual variability in systemic absorption of timolol after ocular application and in its hepatic and renal elimination, but the actual mechanisms remain unknown. Nevertheless, paroxetine appears more sensitive than timolol to variability in CYP2D6 activity, since a larger fraction of its dose undergoes metabolism by CYP2D6 and its autoinhibitory effect may further increase the effect of CYP2D6 genotype.

In a previous study, the maximum heart rate in an exercise test was decreased by at least 20 beats/min in four of 25 patients after ophthalmic application of 0.5% aqueous timolol (Nieminen et al., 2005b). In these four patients, plasma timolol concentration exceeded 0.7 ng/ml. In the present study, only two volunteers had plasma timolol concentrations exceeding 0.7 ng/ml in the placebo phase. In contrast, when paroxetine was given, six volunteers had a C$_{\text{max}}$ over this cutoff value. Furthermore, paroxetine coadministration reduced both the average heart rate and heart rate immediately after upright standing in the orthostatic test after 0.5% timolol application. This suggests that CYP2D6 inhibition by paroxetine can increase the risk of cardiovascular adverse effects in patients using 0.5% aqueous timolol eye drops. In fact, in one case report, severe bradycardia occurred after using both 0.5% timolol eye drops and quinidine, a CYP2D6 inhibitor (Dinai et al., 1985). Similarly, flecainide and propafenone, both known as substrates and inhibitors of CYP2D6, were suggested to interact with ophthalmic timolol, leading to adverse effects, such as bradycardia (Minish and Herd, 2002; Patane et al., 2008).

The present study indicates that plasma timolol levels after using 0.1% timolol hydrogel remain low even when the metabolism of timolol is inhibited by paroxetine; the C$_{\text{max}}$ of timolol varied between 0.056 and 0.322 ng/ml, i.e., below the suggested threshold of 0.7 ng/ml (Nieminen et al., 2005b; Volotinen et al., 2007). These results imply that paroxetine is unlikely to have a clinically significant effect on the benefit-risk ratio of 0.1% timolol hydrogel. The low C$_{\text{max}}$ after the 0.1% hydrogel formulation is partly due to the slow systemic absorption of timolol from this formulation. It should be noted that, in addition, the long half-life of timolol ($\sim 10$ hours versus the true timolol elimination half-life of $\sim 3$–5 hours) with the hydrogel formulation reflects the slow absorption phase, resulting in a “flip-flop” phenomenon. Nevertheless, despite the long half-life with hydrogel formulation, significant systemic accumulation of timolol is unlikely, because the hydrogel is used only once daily. Accordingly, with usual twice-daily application of short-acting 0.5% timolol formulations, the daily systemic exposure to timolol is about 10 times greater than with once daily use of the longer-acting 0.1% formulation.

During all study phases, heart rate declined after timolol administration until the 3-hour assessment, which was followed by lunch (Supplemental Fig. 2), probably mainly because the volunteers adapted themselves to the research facility for the first hours after eye drop application. Interestingly, blood pressures were slightly higher both before and after timolol administration during the paroxetine phase than during the placebo phase, suggesting that paroxetine itself may have
some influence on blood pressure. In any case, it should be noted that the observed pharmacodynamic consequences of the interaction after a single ophthalmic timolol dose in young healthy volunteers may not be fully comparable to those seen in true target populations, which often are older people with cardiovascular or pulmonary diseases, or when other CYP2D6 inhibitors are used.

The results of the present study are clinically relevant, especially among the elderly. Most glaucoma patients are over 60 years old (Quigley, 2011). About 30% of individuals older than 65 years fall at least once a year, and many of the falls occur due to the use of various drugs (Kannus et al., 2005). The use of topical β-adrenoceptor blocking agents increases the risk of falls in elderly glaucoma patients (Glynn et al., 1991). In a recent study, timolol was the most prescribed antiglaucoma medication among about 8700 nursing home residents in Germany, and most residents with glaucoma were concomitantly taking systemic drugs (Huber et al., 2013). The risks related to topically applied β-adrenoceptor blocking agents may be underestimated due to the manner of topical application and unawareness of the potential systemic effects (Higginbotham, 1996).

In conclusion, paroxetine significantly increases plasma timolol concentration after administration of either ophthalmic 0.1% timolol hydrogel or 0.5% aqueous timolol. However, plasma timolol concentrations are several fold higher during the use of the 0.5% formulation than during the use of 0.1% hydrogel. This difference should be kept in mind because timolol, in a concentration-dependent manner, increases the risk of cardiovascular adverse effects, such as bradycardia, conduction disorders, and orthostatic hypotension, especially in elderly glaucoma patients who often have predisposing factors. To minimize these risks, patients should be carefully followed when a concomitant treatment with paroxetine or other strong CYP2D6 inhibitor is considered necessary with 0.5% aqueous timolol. It is likely that such risks are much lower with the 0.1% timolol hydrogel.

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Performed data analysis: Kautiainen, M. Neuvonen, Niemi, Backman.
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