Pharmacokinetic interaction between verapamil and everolimus in healthy subjects

J. M. Kovarik,¹ D. Beyer,¹ M. N. Bizot,¹ Q. Jiang,¹ M. J. Allison² & R. L. Schmouder¹

¹Novartis Pharmaceuticals, Basel, Switzerland; Rueil-Malmaison, France, and East Hanover, NJ and ²MDS Pharma Services, Phoenix, AZ, USA

Correspondence

J. M. Kovarik, Novartis Pharma,
Building WSJ 27.P081, 4002 Basel,
Switzerland.

Tel.: + 41 324 8239

Fax: + 41 324 2959

E-mail:

john.kovarik@pharma.novartis.com

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Aims

We sought to define the influence of verapamil, an inhibitor of CYP3A and P-glycoprotein, on the pharmacokinetics of everolimus, a substrate of this enzyme and transporter.

Methods

This was a two-period, single-sequence, crossover study in 16 healthy subjects. In period 1 subjects received a single 2 mg oral dose of everolimus. In period 2 they received verapamil 80 mg three times daily for a total of 6 days and a single 2 mg dose of everolimus co-administered on the second day of verapamil therapy.

Results

During verapamil co-administration, everolimus C_{max} increased 2.3-fold (90% CI, 1.9, 2.7) from 21 ± 8 to 47 ± 18 ng ml⁻¹ and AUC increased 3.5-fold (90% CI, 3.1, 3.9) from 115 ± 45 to 392 ± 142 ng ml⁻¹ h. Everolimus half-life was only prolonged to a minor extent (32 ± 6 vs. 37 ± 6 h). Verapamil predose concentrations doubled from 32 ± 16 to 74 ± 42 ng ml⁻¹ after single dose administration of everolimus.

Conclusions

Multiple dosing with verapamil increased blood concentrations of everolimus after a single dose by an average 3.5-fold. During verapamil treatment, dose reduction for everolimus should be made guided by blood monitoring and for verapamil by blood pressure monitoring.

Introduction

Everolimus is a macrolide immunosuppressant indicated for prevention of acute rejection episodes after kidney and heart transplantation [1]. Everolimus undergoes extensive metabolism via CYP3A to form several inactive metabolites and is a substrate of P-glycoprotein [1]. Dihydropyridines are calcium channel antagonists used in the treatment of hypertension, arrhythmias, and other cardiovascular disorders in organ transplant patients. In this chemical class, verapamil is a relatively potent inhibitor of P-glycoprotein [2] and a moderate inhibitor of CYP3A [3]. The present study was designed

to define the influence of verapamil on everolimus pharmacokinetics in order to provide guidance for the clinical use of this drug combination.

Methods

Study design

This was a two-period, single-sequence, crossover study in 16 healthy subjects. In period 1 (study days 1–6), subjects received a single 2 mg dose of everolimus on day 1 with pharmacokinetic and clinical evaluations up to day 6. In period 2 (study days 10–16), subjects received verapamil 80 mg three times daily for a total

of 6 days (study days 10–15). On the second day of verapamil, a single 2 mg dose of everolimus was co-administered (study day 11). Pharmacokinetic and clinical evaluations were performed up to day 16.

The protocol was reviewed and approved by the MDS Pharma Services Institutional Review Board (Lincoln, NE, USA) and subjects gave written consent to participate. The study was performed at MDS Pharma Services (Phoenix, AZ, USA).

Drug administration and clinical assessments

On the days of everolimus administration (days 1 and 11), subjects fasted for 10 h overnight and received a 2 mg single oral dose of everolimus (Certican, Novartis) with 240 ml water. Subjects fasted for at least 4 h thereafter. In study period 2, verapamil 80 mg immediate release tablets (Calan, Searle) were administered every 8 h on days 10 to 15 with 240 ml water. Routine laboratory tests were performed the day before each study period (days 0 and 9), 3 days after each everolimus administration (days 4 and 14), and at the completion of the study (day 16). Vital signs were measured the day before each period and daily in period 2. ECG recordings were made at screening, before period 2, and at the end of the study.

Blood sampling

For everolimus, blood samples (5 ml, EDTA) were obtained predose and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36, 48, 60, 72, 96, and 120 h postdose. For verapamil, blood samples (5 ml, EDTA) were obtained before the morning dose on days 11, 12, 14, and 15. Whole blood samples for everolimus and plasma samples for verapamil were transferred to polypropylene tubes and frozen at -20°C .

Drug analysis

Blood everolimus concentrations were determined by liquid chromatography with mass spectrometry as described previously [4]. At the quality control concentrations of 0.90, 20.0, and 40.0 ng ml⁻¹, interassay coefficients of variation were 8.2%, 6.9%, and 9.3% and bias was 1.3%, 9.5%, and 7.4%, respectively. The limit of quantification of everolimus was 0.3 ng ml⁻¹.

Verapamil and the internal standard methoxyverapamil were extracted by a liquid-liquid procedure and analyzed by gas chromatography (Agilent 6890 N network system, column DB5, 15 m $\times$ 0.25 mm $\times$ 0.25 micron, flow rate 2.0 ml min⁻¹, injection volume 1.0 μ l) equipped with a split/splitless capillary injection port (split ratio 1 : 100, injector temperature 340 $^{\circ}\text{C}$) with mass spectrometric detection (Agilent 5973 inert mass

selective detector, manifold temperature 310 $^{\circ}\text{C}$, single ion monitoring). At the quality control concentrations of 5, 198, and 396 ng ml⁻¹, bias was 13.9%, 7.6%, and 4.5%, respectively. Since all plasma samples were analyzed in a single day, calculation of interassay coefficients of variation was not applicable. The assay limit of quantification for verapamil was 2 ng ml⁻¹.

Pharmacokinetic analysis

Standard noncompartmental methods were used to derive the maximum concentration, C_{max} , the time to reach the maximum, t_{max} , the area under the concentration-time curve extrapolated to time infinity, AUC, the elimination rate constant, λ_z , by log-linear regression and the corresponding half-life, $t_{1/2} = \ln(2)/\lambda_z$.

Statistical analysis

An ANOVA was performed on everolimus log-transformed C_{max} , AUC, and half-life with treatment as a fixed effect and subject as a random effect. The results were expressed as the test : reference ratio of the geometric means and 90% confidence interval. Verapamil trough concentrations were compared between day 11 when given alone and days 12–15 when given with everolimus in the above-mentioned ANOVA model.

Results

Table 1 summarizes the pharmacokinetic parameters for everolimus and Figure 1a shows the mean concentration time profiles. Co-administration of verapamil with everolimus increased the C_{max} of everolimus in all 16 subjects ranging from 1.3-fold to 3.8-fold, and by a mean of 2.3-fold (90% CI 1.9, 2.7). Everolimus AUC increased in all 16 subjects in the range 2.2-fold to 6.3-fold and by a mean of 3.5-fold (90% CI 3.1, 3.9). The half-life was essentially unchanged with a mean fold increase of 1.2 (90% CI 1.1, 1.2).

Figure 1b shows that verapamil trough concentrations doubled after the single dose of everolimus from 32 ± 16 to 74 ± 42 ng ml⁻¹ (fold increase of 2.3 (90% CI 2.1, 2.4)). Mean systolic/diastolic supine blood pressure averaged $120 \pm 8/69 \pm 6$ mmHg on day 9 before verapamil. On days 11 and 12 when verapamil trough concentrations doubled, values decreased only slightly from $106 \pm 9/62 \pm 6$ mmHg to $104 \pm 9/61 \pm 7$ mmHg. Values returned to near baseline on day 16 ($116 \pm 11/64 \pm 6$ mmHg). The total area under the blood pressure vs. time curve was not correlated with the AUC of verapamil over the period from day 9–15. For diastolic blood pressure the slope was -0.07 ($P = 0.394$) and for systolic blood pressure the slope was -0.09 ($P = 0.508$).

When verapamil trough concentrations on day 14

Table 1

Everolimus pharmacokinetic parameters

Parameter	Everolimus alone	Everolimus with verapamil
$t_{\max}$ (h)	0.5 (0.5–1.5)	0.5 (0.5–1.5)
$C_{\max}$ (ng ml ⁻¹)	21 ± 8	47 ± 18
AUC (ng ml ⁻¹ h)	115 ± 45	392 ± 142
$t_{1/2}$ (h)	32 ± 6	37 ± 6

Values are mean ± SD except for $t_{\max}$ which is median (range). $t_{\max}$ is time to reach maximum concentration; $C_{\max}$ is maximum concentration; AUC is area under the concentration-time curve; $t_{1/2}$ is the half-life.

before everolimus co-administration were regressed against the fold-increase in everolimus AUC, the slope (0.006 fold-increase per ng ml⁻¹ verapamil) was not significantly different from zero ($P = 0.728$). Hence, verapamil exposure was not predictive of the magnitude of the interaction affecting everolimus.

Discussion

There was a consistent increase in the $C_{\max}$ and AUC of everolimus during co-administration of verapamil, whereas everolimus half-life was essentially unaltered. Together these observations suggest that verapamil primarily increases the bioavailability of everolimus, rather than decreasing its systemic clearance, probably through an interaction with CYP3A and P-glycoprotein. A proposed drug interaction classification for drugs metabolized by CYP3A categorizes a mean increase in AUC of <2.0-fold as weak, 2.0–4.9-fold as moderate, and ≥5.0-fold as strong [3]. Accordingly, the 3.5-fold average increase in everolimus AUC in the presence of verapamil would be classified as a moderate interaction.

Given a mean half-life of 4 h for verapamil, we assumed trough concentrations would be at steady state after three doses and chose to co-administer everolimus with the fourth verapamil dose. If verapamil was at steady state on day 11, then the doubling of verapamil concentrations we observed on day 12 would be caused by everolimus. If verapamil was not at steady state, this increase in concentrations may represent the continued accumulation of verapamil independent of everolimus co-administration. The data do not allow a firm conclusion to be made regarding the mechanism of this increase since only one verapamil trough concentration was obtained before everolimus co-administration. In

Figure 1

(A) Mean everolimus concentration-time profiles after administration alone (○) and with verapamil (●). Bars represent the 95% confidence intervals. (B) Mean verapamil trough concentrations (●) and individual concentrations (lines). Everolimus was administered immediately after the time of the verapamil trough concentration on study day 11

general, everolimus has a low potential to inhibit CYP3A as demonstrated by lack of effect on atorvastatin pharmacokinetics [5], which is mainly metabolized by CYP3A [3]. However, the trough verapamil concentrations we measured on day 11 are in the range reported by others for steady state values [6, 7]. Hence, a conservative interpretation of our data is that everolimus increased exposure to verapamil. The observed increase in verapamil concentration was associated with only a minor change in supine blood pressure in these healthy subjects.

The data from this study, albeit in healthy subjects, might serve as a guide to clinicians using this drug combination. Organ transplant patients starting with the recommended everolimus dose of 0.75 mg twice daily are generally titrated via blood monitoring to a mean long-term dose of 0.9 mg twice daily [8]. Given the available everolimus tablet strengths (0.25, 0.5, 0.75,

1 mg), appropriate dose reductions can be made to compensate for the interaction with verapamil. The precise everolimus dose adjustment for a given patient should be determined by blood level and clinical monitoring. Whether an increase of 2.3-fold in verapamil plasma concentrations is clinically relevant for patients could be assessed by blood pressure monitoring with subsequent verapamil dose adjustment if warranted.

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Competing interests: JMK, DB, MNB and RLS have shares in Novartis.

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