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PHARMACOKINETICS AND DISPOSITION

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Effect of roxithromycin on the pharmacokinetics of lovastatin in volunteers

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Abstract *Objective*: To investigate the influence of concomitant administration of roxithromycin on the plasma pharmacokinetics of lovastatin.
Methods: In an open, randomized, crossover study, 12 healthy volunteers received 80 mg lovastatin orally either alone or concomitantly with 300 mg roxithromycin after 5-day pretreatment with roxithromycin 300 mg daily. Plasma concentrations of lovastatin (lactone and acid) were determined using high-performance liquid chromatography, and the pharmacokinetic parameters were estimated.
Results: The mean (±SD) pharmacokinetic parameters of lovastatin lactone with and without roxithromycin were maximum concentration (C_{max}) 8.49 ± 6.80/16.3 ± 9.4 ng ml^{-1}, time to C_{max} (t_{max}) 1.8 ± 0.4/1.7 ± 0.6 h, terminal plasma half-life ($t_{1/2}$) 4.3 ± 2.0/3.7 ± 2.5 h, area under the plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$) 53 ± 60/85 ± 67 ng ml^{-1} h. The respective parameters of lovastatin acid were C_{max} 24.6 ± 13.4/17.8 ± 11.0 ng ml^{-1}, t_{max} 3.7 ± 1.1/4.1 ± 0.7 h, $t_{1/2}$ 3.2 ± 2.5/4.3 ± 2.8 h, $AUC_{0-\infty}$ 149 ± 123/105 ± 58 ng ml^{-1} h. Mean bioavailability of lovastatin lactone was lower and that of lovastatin acid was higher with concomitant treatment. However, the differences were significant only with respect to lovastatin lactone (AUC and C_{max}) and C_{max} of lovastatin acid.
Conclusion: Roxithromycin does not influence the pharmacokinetics of lovastatin in such a way that dosage adjustment of lovastatin seems to be necessary during co-administration.

Keywords Lovastatin · Roxithromycin · Pharmacokinetics

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Introduction

Chronic coronary artery disease and acute myocardial infarction have been associated with serological evidence of *Chlamydia pneumoniae* infection in several studies. In addition, infection with *C. pneumoniae* induces a marked increase in cholesteryl ester synthesis in vitro in human monocyte-derived macrophages incubated with low-density lipoproteins, producing the foam cells that are characteristic of early atherosclerosis. Serological evidence of previous *C. pneumoniae* infection is also associated with a proatherogenic serum cholesterol profile. Therefore, cholesterol-lowering therapy may be accompanied with antibiotic therapy directed against *C. pneumoniae*, e.g., with macrolides [1, 2].

Inhibitors of cytochrome P_{450} (CYP)3A4 metabolism such as itraconazole [3, 4], grapefruit juice [5], or the macrolide erythromycin [6] greatly increase the bioavailability of the hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors lovastatin and/or simvastatin, and increased plasma drug levels have been regarded as an index of potential untoward effects such as severe skeletal muscle toxicity and rhabdomyolysis [7]. Rhabdomyolysis associated with the concurrent use of simvastatin or lovastatin and macrolides have been observed with erythromycin [8, 9], but also with the new macrolides clarithromycin and azithromycin [10]. Therefore, the aim of the present study was to investigate the influence of roxithromycin on the pharmacokinetics of lovastatin.

Materials and methods

Drugs and chemicals

Lovastatin lactone and simvastatin lactone were provided by Merck Sharp and Dohme (MSD, Munich, Germany). The corresponding acids were obtained by means of alkaline hydrolysis of the lactones according to a procedure provided by the manufacturer. Lactone (8 mg; lovastatin or simvastatin) was dissolved in 0.2 ml ethanol. NaOH (0.3 ml 0.1 M) was added. The mixture was heated for 2 h at 50°C, then neutralized to a pH of approximately 7.2 and brought to a volume of 10 ml with distilled water. Aliquots of the resulting stock solutions of lovastatin acid or simvastatin acid were stored frozen. 1-Bromo acetylpyrene was purchased from Aldrich (Steinheim, Germany), disposable BondElut columns from Phenomenex (Aschaffenburg, Germany), and acetonitrile and methanol [high-performance liquid chromatography (HPLC) grade] from Baker (Gross-Gerau, Germany). All other chemicals were of analytical grade and obtained from Merck (Darmstadt, Germany).

Subjects

Twelve volunteers (6 males, 6 females, 24–34 years, median 27 years, body mass index 19–27 kg m^{-2}, median 22 kg m^{-2}) were enrolled into the study. They were healthy, as assessed by medical history, physical examination, and routine blood and urine chemistry including serum creatine kinase activity. One subject was a smoker; five female subjects were using contraceptive steroids. The study protocol was approved by the ethics committee of the Bavarian Medical Association, Munich, Germany. Written informed consent was obtained from all subjects.

Study design

The study was performed according to an open, blockwise, randomized, crossover design. The subjects received 80 mg lovastatin (two tablets Mevinacor 40 mg, MSD) orally, either alone or concomitantly with 300 mg roxithromycin (1 tablet Rulid 300 mg, Aventis, Bad Soden, Germany) after 5-day pretreatment with roxithromycin 300 mg daily separated by a washout period of 2 weeks. Roxithromycin was administered on an empty stomach 15 min before breakfast, lovastatin immediately after breakfast. A standardized lunch was served after 4.5 h, a snack after 7 h, and dinner after 10 h. Smoking, alcoholic or caffeine-containing beverages were not allowed from 12 h before until 24 h after drug administration. Use of no other medications, besides oral contraceptives, was permitted during the course of the study.

Blood sampling schedule and drug assays

Venous blood samples were drawn into heparinized tubes predose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 h after lovastatin administration. Additional blood samples were drawn just before the morning dose on day 5 during the period with roxithromycin treatment. Plasma was obtained by centrifugation (10 min, 2300 *g*) and stored at –70°C until assay. Lovastatin lactone and lovastatin acid were assayed in plasma using HPLC with fluorimetric detection adapting a published method [11]. Plasma (1 ml) was spiked with simvastatin lactone and simvastatin acid as internal standards. Then, the lactones and the acids were separated on disposable Bond-Elut C8 extraction columns. Lovastatin acid and simvastatin acid were derivatized to fluorescing compounds with 1-bromo acetylpyrene in acetonitrile, the lactones were derivatized after alkaline hydrolysis to the acids. The derivatives were further purified on a BondElut PBA column followed by a BondElut C18 column and separated on a Hypersil ODS 3-μm column (125×4 mm I.D., Bischoff, Leonberg, Germany) with acetonitrile–water (80:20, v/v) as eluent. The retention time of derivatized lovastatin (flow rate 1 ml/min, column temperature 30°C) was 7.9–8.1 min and of simvastatin 10.2–10.6 min. The limit of quantification was 500 pg/ml lovastatin acid and 250 pg/ml lovastatin lactone. The method was validated over 0.25–25 ng ml^{-1} for lovastatin lactone and 0.5–25 ng ml^{-1} lovastatin acid. Intra- and inter-assay precision and accuracy were better than 13% as determined using spiked plasma samples over the range of 2.4–17 ng ml^{-1} (lactone) and 4.8–33 ng ml^{-1} (acid). Roxithromycin was assayed according to a published method [12].

Pharmacokinetic analysis and statistical evaluation

The pharmacokinetic parameters of lovastatin lactone and lovastatin acid were assessed using standard non-compartmental methods. The area under the plasma concentration–time curve to the last quantifiable concentration ($AUC_{0-tlast}$) was calculated using the linear trapezoidal rule. The elimination constant λ_z was calculated using log-linear regression in the terminal elimination phase. The terminal half-life ($t_{1/2}$) was calculated according to $t_{1/2} = \ln 2/\lambda_z$. The measured last concentration (C_{last}) was used for extrapolation to infinity to determine $AUC_{0-\infty} = AUC_{0-tlast} + C_{last}\lambda_z$. The pharmacokinetic parameters were compared using the Wilcoxon matched-pairs signed-ranks test assuming $P < 0.05$ as significant. In addition, the dosage regimens lovastatin/roxithromycin (test) and lovastatin (reference) were tested for bioequivalence. The range of bioequivalence was set to 70–143% maximum concentration (C_{max}) and 80–125% (AUC) using logarithmically transformed data. The no-difference hypothesis in time to C_{max} (t_{max}) was accepted, if the 90% confidence interval of the differences in t_{max} included the value "zero" [13].

Results

Plasma concentrations and pharmacokinetic parameters

The trough levels of roxithromycin on day 5 and day 6 of dosage were 2.9 ± 1.6 μg/ml and 2.8 ± 1.5 μg/ml, respectively. The individual concentrations on day 6 compared with day 5 were $102 \pm 15\%$, indicating reliable bioavailability of roxithromycin. Mean plasma concentrations of lovastatin lactone were lower, and those of lovastatin acid were higher, with concomitant treatment (Fig. 1). However, the differences were significant only with respect to lovastatin lactone (AUC and C_{max}) and C_{max} of lovastatin acid (Table 1). Both treatments were bioinequivalent with respect to lovastatin lactone. Proof of bioequivalence with respect to lovastatin acid failed, because one subject (no. 8, male, non-smoker) showed outlying results, namely eightfold higher C_{max} (35.1 vs 4.3 ng ml^{-1}, Fig. 2) and 6.5-fold higher AUC (482 vs 74 ng ml^{-1} h, zFig. 3) of lovastatin acid during comedication of roxithromycin than lovastatin alone. Also the half-lives of lovastatin lactone (6.1 vs 9.4 h during lovastatin with roxithromycin vs lovastatin alone) and lovastatin acid (10.5 vs 10.3 h) were higher in these subjects than the other subjects. The kinetic parameters of subjects using oral contraceptives did not differ from those of the other subjects (Fig. 2 and Fig. 3).

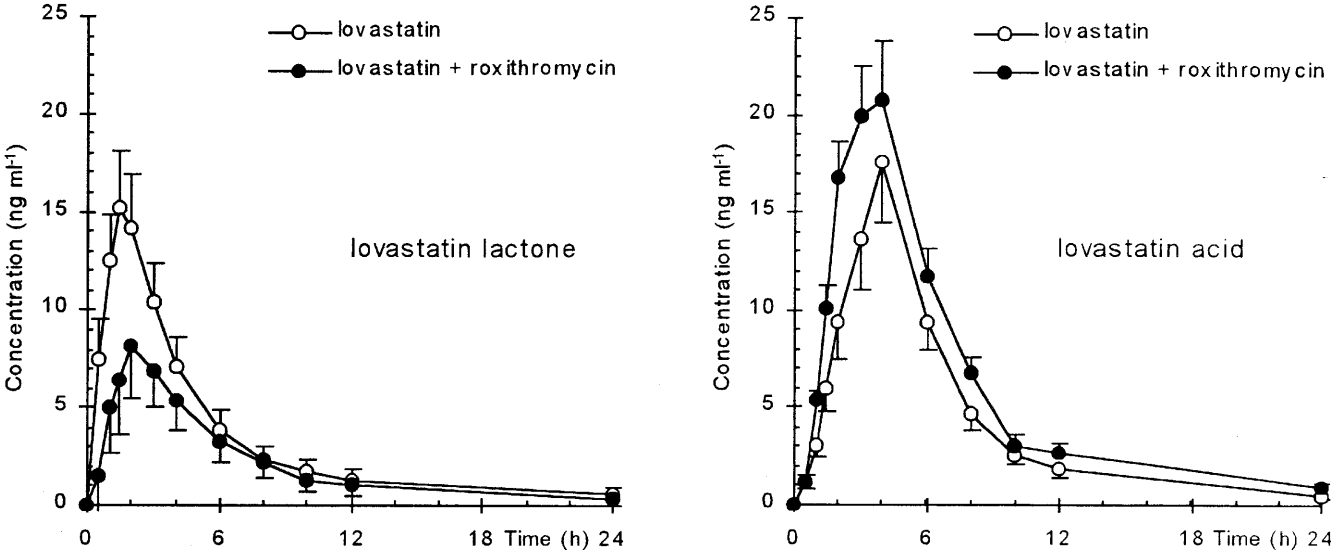

Fig. 1 Mean (±SEM) plasma concentrations of lovastatin (lactone and acid) in 12 healthy volunteers following oral administration of 80 mg lovastatin alone (*open circles*) or with 300 mg roxithromycin concomitantly (*closed circles*)

Discussion and conclusions

The aim of the study was to assess the influence of roxithromycin on the pharmacokinetics of lovastatin. Therefore, the subjects were treated with one single 80-mg dose of lovastatin alone and after a 5-day pretreatment with roxithromycin to get steady-state conditions. This design differs from the situation in medical practice where typically lovastatin is administered continuously and roxithromycin may be added for a few days, and it does not reveal the possible influence of roxithromycin on the pharmacodynamics, i.e., the lipid-lowering effect, of lovastatin that is observed only after multiple lovastatin dosing.

Our study demonstrated that in general there is no risk of accumulation of lovastatin when roxithromycin is co-administered. The plasma concentrations of the active entity lovastatin acid were only marginally elevated, the concentrations of the parent compound lovastatin lactone were even lower than lovastatin alone. Proof of bioequivalence with respect to the active entity lovastatin acid failed because one subject (no. 8, Fig. 2 and Fig. 3) showed contrasting results, namely considerably higher plasma concentrations of both lovastatin lactone and lovastatin acid with co-administered roxithromycin. However, only peak concentrations and AUCs increased, but not $t_{1/2}$s. Therefore, the interaction is a first-pass rather than a systemic effect, similar to the interaction of itraconazole [4] or grapefruit juice [5] with lovastatin or simvastatin. Accordingly, the influence should be lower [14] when roxithromycin is administered in the morning and lovastatin in the evening as suggested by the manufacturer. Moreover, the plasma concentrations after administration of lovastatin alone were low to very low in this subject compared with the values in the other subjects (Fig. 2). Therefore, high day-to-day variability of the bioavailability of lovastatin may have contributed additionally to the conspicuous results

Table 1 Mean (±SD) pharmacokinetic parameters of lovastatin lactone and lovastatin acid in 12 healthy volunteers following oral administration of 80 mg lovastatin lactone alone (lova, reference) and with concomitant roxithromycin 300 mg daily (lova/roxi, test) and test for bioequivalence. C_{max} maximum concentration, t_{max} time to C_{max}, $t_{1/2}$ terminal plasma half-life, $AUC_{0-\infty}$ area under the plasma concentration–time curve from zero to infinity, *P.E.* point estimate, *C.I.* confidence interval

Parameter	Lova/roxi	Lova	P.E. (90% C.I.)
Lovastatin lactone			
C_{max} (ng ml^{-1})	8.49 ± 6.80*	16.3 ± 9.4*	0.45 (0.35, 0.64)
t_{max} (h)	1.8 ± 0.4	1.7 ± 0.6	0.25 (–0.25, 0.50)
$t_{1/2}$ (h)	4.3 ± 2.0	3.7 ± 2.5	
$AUC_{0-\infty}$ (ng ml^{-1} h)	53 ± 60*	85 ± 67*	0.52 (0.46, 0.61)
Lovastatin acid			
C_{max} (ng ml^{-1})	24.6 ± 13.4*	17.8 ± 11.0*	1.29 (1.05, 1.63)
t_{max} (h)	3.7 ± 1.1	4.1 ± 0.7	–0.5 (–1.00, 0.00)
$t_{1/2}$ (h)	3.2 ± 2.5	4.3 ± 2.8	
$AUC_{0-\infty}$ (ng ml^{-1} h)	149 ± 123	105 ± 58	1.16 (0.94, 1.50)

*$P < 0.05$

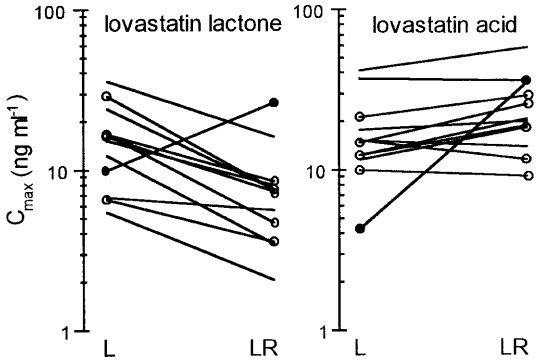

Fig. 2 Individual variations in lovastatin lactone and lovastatin acid peak concentration (C_{max}) values during 80 mg lovastatin alone (*L*) and lovastatin plus 300 mg roxithromycin (*LR*). The values of subjects using oral contraceptives are marked with an *open circle*, those of subject no. 8 with a *closed circle*. (Note the logarithmic scale)

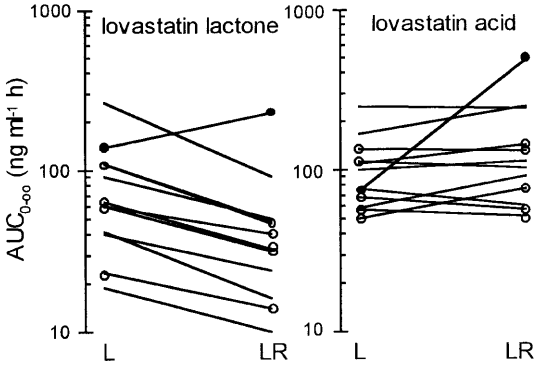

Fig. 3 Individual variations in lovastatin lactone and lovastatin acid area under the plasma concentration–time curve from 0 to infinity ($AUC_{0-\infty}$) values during 80 mg lovastatin alone (*L*) and lovastatin plus 300 mg roxithromycin (*LR*). The values of subjects using oral contraceptives are marked with an *open circle*, those of subject no. 8 with a *closed circle*. (Note the logarithmic scale)

considering the generally low absorption rate (30%) and poor bioavailability (5%) of lovastatin [7].

It is assumed that lovastatin lactone is subject to CYP3A4 oxidation, rather than its active open-acid form, which in turn is in equilibrium with the lactone form [7, 15]. Therefore, inhibitors of lovastatin metabolism should exert the same influence on the plasma concentrations of the lactone and the acid. This has been confirmed in previous studies with lovastatin and its methyl derivative simvastatin [7]. However, the results presented here, unaltered or marginally increased plasma concentrations of lovastatin acid and significantly lower plasma concentrations of lovastatin lactone, are not compatible with this concept. We cannot provide a convincing explanation for our contrasting results. However, the bioavailability of lovastatin seems to be sensitive to gastrointestinal transit time, because the bioavailability is 50% higher when lovastatin is administered after food [7], i.e., when gastrointestinal transit time is prolonged. Therefore, faster transit through the small intestine during concomitant roxithromycin administration and accordingly smaller absorption rate could explain the lower plasma concentrations of lovastatin lactone, even though acceleration of gastrointestinal transit is typical for erythromycin and its acidic degradation products, but not for the new macrolides including roxithromycin [16, 17]. Also an influence of gastrointestinal transit time on the bioavailability of an HMG-CoA inhibitor was reported recently [18]. In that study, the plasma concentrations of simvastatin lactone were unaltered and the plasma concentrations of simvastatin acid were decreased with co-administered cisapride. The results were explained by impaired enterohepatic reabsorption of simvastatin acid and hence decreased plasma concentrations. The different influence of a shortened gastrointestinal transit time on the plasma concentrations of lovastatin compared with simvastatin may be due to the fact that the absorption rate of simvastatin is better compared with lovastatin (70–80% vs 30%) and not enhanced after food [7].

To explain the relatively higher plasma concentrations of lovastatin acid with co-administered roxithromycin we assume that roxithromycin, which is only a poor CYP3A4 inhibitor when compared with erythromycin [19], is able to inhibit the hydroxylation of lovastatin acid to some extent but not of lovastatin lactone which has a much higher affinity to the enzyme than the acid form [20].

We summarize that concomitant administration of roxithromycin does not increase the plasma concentrations of lovastatin lactone and increases – if at all – only marginally the plasma concentrations of lovastatin acid. In order to minimize any risk of interaction at the absorption site, the time of daily oral roxithromycin and lovastatin administration should be separated as much as possible, and clinical symptoms of myopathy or increasing serum creatine phosphokinase should be monitored carefully.

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