In vivo inhibition profile of cytochrome P450TB (CYP2C9) by (±)-fluvastatin

Background: (±)-Fluvastatin is a synthetic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor that selectively and competitively inhibits P450TB (CYP2C9) in vitro. The potential for kinetic interactions in vivo between fluvastatin and P450TB substrates was therefore investigated in healthy volunteers.

Methods: Diclofenac (25 mg orally) oxidation was used as a marker of P450TB activity on days 0, 1, and 8 of fluvastatin treatment (40 mg/day).

Results: Diclofenac peak concentration (C_{max}) increased over time (0.28 [SD, 0.12], 0.38 [0.20], and 0.45 [0.4] mg/L on days 0, 1, and 8, respectively). Oral clearance was reduced on days 1 and 8 (14% and 15%, respectively). A time-dependent decrease in urinary metabolic ratio (MR, 4′-hydroxydiclofenac/diclofenac) was noted (1.07 [0.34], 0.90 [0.23] and 0.70 [0.18] on days 0, 1, and 8, respectively [$p < 0.0001$]) for the first 4 hours. The interaction was clear in only some individuals; MR reduction was related to baseline MR and it was more pronounced in subjects with a higher baseline MR ($p < 0.01$). Fluvastatin C_{max} (0.18 [0.11] and 0.32 [0.1] mg/L on days 1 and 8, respectively) and area under the curve (0.28 [0.12] and 0.43 [0.15] hr · mg/L on days 1 and 8, respectively; $p < 0.001$) increased over time. Diclofenac MR reduction was correlated with fluvastatin concentrations.

Conclusion: Interactions between fluvastatin and P450TB substrates (phenytoin, oral anticoagulants, oral hypoglycemic agents, and nonsteroidal antiinflammatory drugs) may occur, at least in some patients. (Clin Pharmacol Ther 1995;58:412-7.)

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(±)-Fluvastatin ([*R**,*S**,(*E*)]-(±)-sodium-3,5-dihydroxy-7-[3(4-fluorophenyl)-1-(1-methylethyl)-1*H*-indol-2-yl]-hept-6-enoate) is a new hypolipidemic agent that, like lovastatin, simvastatin, and pravastatin, inhibits the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis. It has been developed as a mixture of two erythrostereoisomers, although most of the inhibiting activity resides in one enantiomer, the 3*R*,5*S* configuration [(+)-fluvastatin]. (±)-Fluvastatin is rapidly and almost completely (98%) absorbed from the gastrointestinal tract, but its bioavailability is only 25% (for a dose of 10 mg) because of extensive first-pass metabolism. It is highly protein bound (99%). Fluvastatin is almost completely metabolized in the liver (hydroxylation, *N*-dealkylation, and β-oxidation) before fecal excretion, its major route of elimination (>90%).[1]

In vivo interactions with oral anticoagulants (acenocoumarol and warfarin) have been reported with lovastatin[2] and simvastatin.[3,4] Displacement from protein binding was shown not to be responsible for the lovastatin-warfarin interaction,[5] and the most likely causal mechanism of these interactions is through inhibition of cytochrome P450-catalyzed elimination in the liver. The likelihood of similar interactions with (±)-fluvastatin, which has a different chemical structure, was first evaluated in vitro by comparison of its inhibiting potency for P450TB, (CYP2C9, the cytochrome responsible for oxidation of nonsteroidal antiinflamatory drugs and oral anticoagulants[6-8]) to those of lovastatin, pravastatin, and simvastatin. These studies showed that (±)-fluvastatin has a greater in vitro affinity for P450TB than the other HMG-CoA reductase inhibitors.[9] Simulations with quantitative models that integrateg in vitro inhibition data and in vivo

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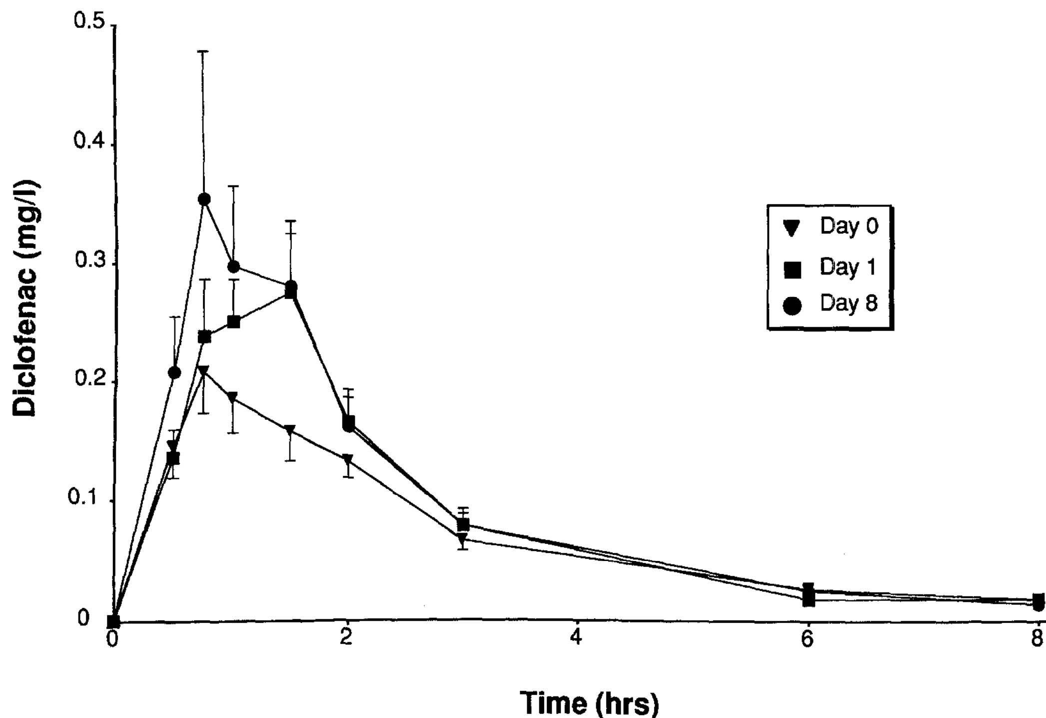

Fig. 1. Mean ± SEM diclofenac plasma concentrations on days 0, 1, (start of fluvastatin) and 8 (1 week of fluvastatin treatment); $n = 14$.

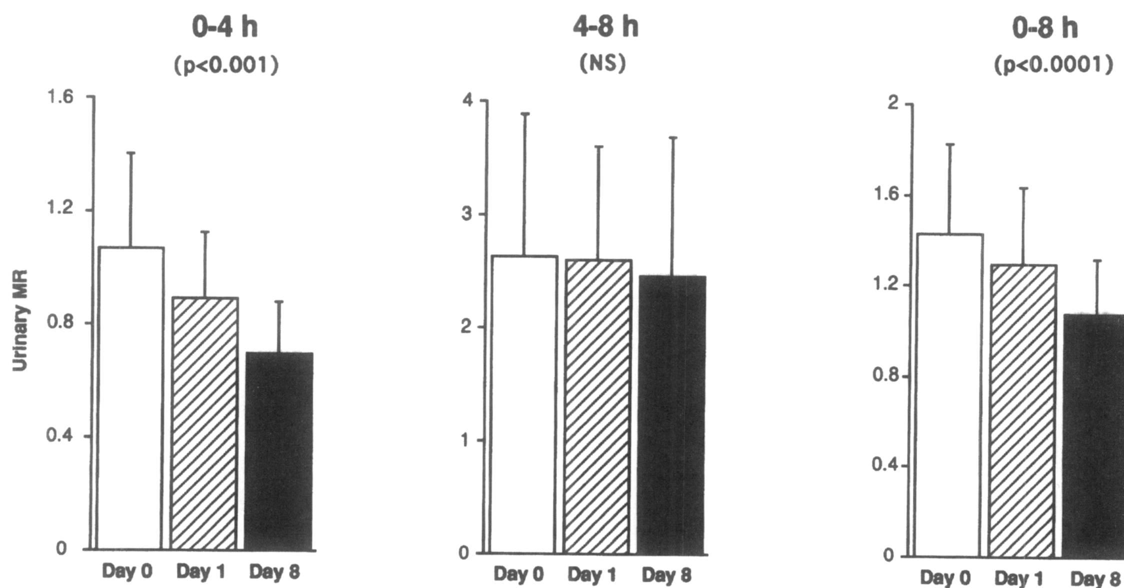

Fig. 2. Mean ± SD fractionated and cumulative urinary diclofenac metabolic ratios (MR; 4′-hydroxydiclofenac/diclofenac) on days 0, 1, and 8; $n = 14$.

Table I. Mean ± SD pharmacokinetic parameters for diclofenac and fluvastatin (n = 14)

	Diclofenac						
Day	C_{max} *(mg/L)*	$t_{1/2}$ *(hr)*	*AUC(0−∞) (hr · mg/L)*	CL_{oral} *(L/hr/kg)*	*MR(0-4 hr)*	*MR(4-8 hr)*	*MR(0-8 hr)*
0	0.28 ± 0.12	1.83 ± 1.74	0.63 ± 0.21	0.70 ± 0.27	1.07 ± 0.34	2.63 ± 1.26	1.43 ± 0.40
1	0.38 ± 0.20	1.29 ± 0.79	0.70 ± 0.19	0.60 ± 0.19	0.90 ± 0.23	2.60 ± 1.00	1.30 ± 0.34
8	0.45 ± 0.41	1.21 ± 0.56	0.79 ± 0.51	0.59 ± 0.20	0.70 ± 0.18	2.46 ± 1.22	1.07 ± 0.25
p Value	0.29*	0.15*	0.06†	0.06†	<<0.01*	0.85*	<<0.01*

*ANOVA; †Friedman test; ‡*t* test.

pharmacokinetic data suggested that interactions with P450TB substrates were unlikely, unless mechanisms other than simple competitive inhibition [irreversible binding to P450TB) or those that result in higher unbound concentrations (such as reduced clearance or plasma protein binding, (±)-fluvastatin or metabolite accumulation in hepatocytes] contribute.[10]

The goal of this study was to evaluate the actual potential for in vivo (±)-fluvastatin interactions with P450TB substrates in human volunteers with use of diclofenac oxidation as a marker of P450TB activity.[11]

METHODS

Drugs

Capsules of (±)-fluvastatin (40 mg capsule, batch X067 0792) and its desisopropylpropionic acid derivative were provided by Sandoz Pharma (Basel, Switzerland). Diclofenac was administered as a liquid formulation (Voltaren drops, Ciba-Geigy, Basel, Switzerland).

Subjects

Fourteen healthy volunteers (seven women and seven men) were selected after medical examination. They did not have ulcers, asthma, or any allergy to drugs. They had not taken any drug for 2 weeks before the study; the women were taking oral contraceptives. Their average (±SD) age was 27 ± 4 years, their body weight was 65 ± 8 kg, and their height was 173 ± 8 cm. The investigational protocol was approved by the institutional ethical committee (Department of Medicine, University of Geneva), and all subjects gave written informed consent.

Protocol

Fasting volunteers received 25 mg oral doses of diclofenac at 10 am on days 0, 1, and 8. (±)-Fluvastatin (one 40 mg capsule) was administered from day 1 to day 8 at 9 am. A standardized lunch was given 2 hours after diclofenac administration. Blood samples were collected on days 0, 1, and 8 before fluvastatin administration, before taking diclofenac, and ½, ¾, 1, 1½, 2, 3, 6, and 8 hours after diclofenac medication. Blood samples were centrifuged for 10 minutes at 3000 rpm and plasma was stored at −20° C until analyzed. Urine was also collected over 8 hours (0 to 4 and 4 to 8 hours) and stored at −20° C until analyzed to determine the urinary metabolic ratio: MR = 4′-hydroxydiclofenac/diclofenac.

Analytic methods

Plasma diclofenac. Mefenamic acid (50 μl of a 20 μmol/L solution), the internal standard, was added to aliquots of plasma samples (500 μl). The samples were acidified with 2 mol/L hydrochloric acid (200 μl) and extracted with dichloromethane (5 ml). Samples were tumbled for 10 minutes and then centrifuged at 5000 rpm for 8 minutes. The organic layer (4 ml) was transferred to a clean tube and evaporated to dryness under a nitrogen stream.[12] The residue was reconstituted in the mobile phase (75:25 mixture of a 0.1 mol/L, pH 7.4 phosphate buffer and acetonitrile with 0.02% triethylamine; 250 μl). Supernatants (100 μl) were injected (HP 1050 autosampler, Hewlett-Packard Co., Roseville, Calif.) directly onto a Lichrocart C_{18} (E. Merck, Darmstadt, Germany) column (50 × 4 mm, 4 μm) protected by a precolumn (4 × 4 mm, 4 μm). The mobile phase was delivered at 1 ml/min by a HP 1050 pump (Hewlett-Packard Co.) and the column effluent was monitored at 282 nm (HP 1050 variable wavelength ultraviolet detector, Hewlett-Packard Co.). Peak heights (HP 3396A integrator, Hewlett-Packard Co.) were used for quantification. (±)-Fluvastatin or its metabolite(s) did not interfere with the assay.

Urinary diclofenac and 4′-hydroxydiclofenac. Ascorbic acid (100 μl of a 400 mg/L solution) and 5 mol/L sodium hydroxide (100 μl) were added to urinary samples (100 μl), and the mixture was vortexed for 30 seconds and heated for 1 hour at 75° C. Thereafter, mefenamic acid (50 μl of a 20 μmol/L solution), the internal standard, was added and samples were

Fluvastatin	
C_{max} (*mg/L*)	*AUC(0-9)* (*hr · mg/L*)
0.18 ± 0.11	0.28 ± 0.12
0.32 ± 0.10	0.43 ± 0.15
<<0.01‡	<<0.01‡

acidified with 2 mol/L hydrochloric acid (500 μl).[13] The remainder of the extraction procedure was the same as that described for plasma. The residues were reconstituted in the mobile phase (80:20 mixture of 0.1 mol/L, pH 7.4 phosphate buffer and acetonitrile; 500 μl) and diclofenac and 4′-hydroxydiclofenac were detected as described for plasma samples. There was no interference from (±)-fluvastatin or its metabolite(s).

Plasma fluvastatin. Fluvastatin was quantified by means of an HPLC method with fluorescence detection as described.[14]

Statistical analysis

Pharmacokinetic parameters for diclofenac alone after single and multiple doses of (±)-fluvastatin were compared by use of ANOVA and the Friedman test (nonparametric). Pharmacokinetic parameters for fluvastatin and its desisopropylpropionic acid derivative were compared by use of the paired *t* test.

RESULTS

The influence of (±)-fluvastatin on diclofenac pharmacokinetic parameters is shown in Table I. Peak concentration (C_{max}) increased in the presence of fluvastatin, and this effect is dependent on the duration of fluvastatin treatment (Fig. 1). The area under the curve (AUC) was also increased by fluvastatin treatment ($p < 0.1$, Friedman test), and oral clearance was reduced on days 1 and 8 (by 14% and 15%, respectively). On the other hand, (±)-fluvastatin had no effect on diclofenac half-life. A decrease in urinary MR (4′-hydroxydiclofenac/diclofenac) was noted during the first 4 hours ($p < 0.001$, ANOVA) but not from the fourth to the eighth hour after diclofenac intake ($p = 0.85$, ANOVA). The MR was also reduced when 0- to 4-hour and 4- to 8-hour samples were pooled (Fig. 2). The reduction of MR was more pronounced in subjects with a higher baseline MR ($p = 0.0004$ on day 1 and $p = 0.003$ on day 8, Fisher's *r* to *z* transformation; Fig. 3).

Fluvastatin pharmacokinetic parameters are shown in Table I. C_{max} was higher on day 8 than on day 1 (Fig. 4; $p = 0.005$, *t* test). The AUC and the ratio of plasma clearance to bioavailability (CL/*F*) were also increased on day 8 ($p = 0.0007$ and 0.0009 respectively, *t* test).

The desisopropylpropionic acid derivative of fluvastatin (or metabolite 8) is present on days 1 and 8, with a significantly higher concentration on day 8 ($p = 0.044$ for C_{max}, $p = 0.002$ for AUC, *t* test; Fig. 4).

The reduction of diclofenac MR was correlated with fluvastatin pharmacokinetic parameters; it was more pronounced on days 1 and 8 in subjects with high fluvastatin AUC ($p = 0.014$ on day 1 and $p = 0.064$ on day 8, Fisher's *r* to *z* transformation). On the other hand, the reduction of diclofenac MR was not statistically correlated with the pharmacokinetics of fluvastatin metabolite, although on day 8 the reduction was correlated with high fluvastatin metabolite AUC values ($p = 0.047$, Fisher's *r* to *z* transformation).

DISCUSSION

This study shows that fluvastatin inhibits diclofenac oxidation, although in only some individuals. The increase in diclofenac C_{max} is not statistically significant perhaps because of highly variable absorption in some individuals. However, the decrease of the urinary diclofenac MR over the first 4 hours (the most sensitive parameter) is highly significant. Diclofenac inhibition is most likely transient because the urinary MR decreases only over the first 4 hours. In addition, it must be noted that MR reduction is more pronounced in subjects with higher baseline MR values. Therefore, fluvastatin administered 1 hour before oral intake of diclofenac seems to inhibit only the hepatic first-pass biotransformation of diclofenac.

Contrary to previous reports,[1] both fluvastatin and the desisopropylpropionic acid metabolite accumulate in plasma during long-term administration. In parallel, the inhibition of diclofenac metabolism is more pronounced during multiple-dose administration than after a single dose of fluvastatin, and the reduction of MR is dependent on fluvastatin concentrations but not on those of the metabolite except on day 8. The accumulation of fluvastatin and possibly also its metabolite and/or other metabolites therefore seem to play a role on P450TB inhibition.

These results are not in total agreement with predictive simulations of in vivo inhibition profiles from in vitro data, which suggested that, if present, the inhibition of P450TB was expected to be transient, as observed in this study,[9] but that interactions with P450TB

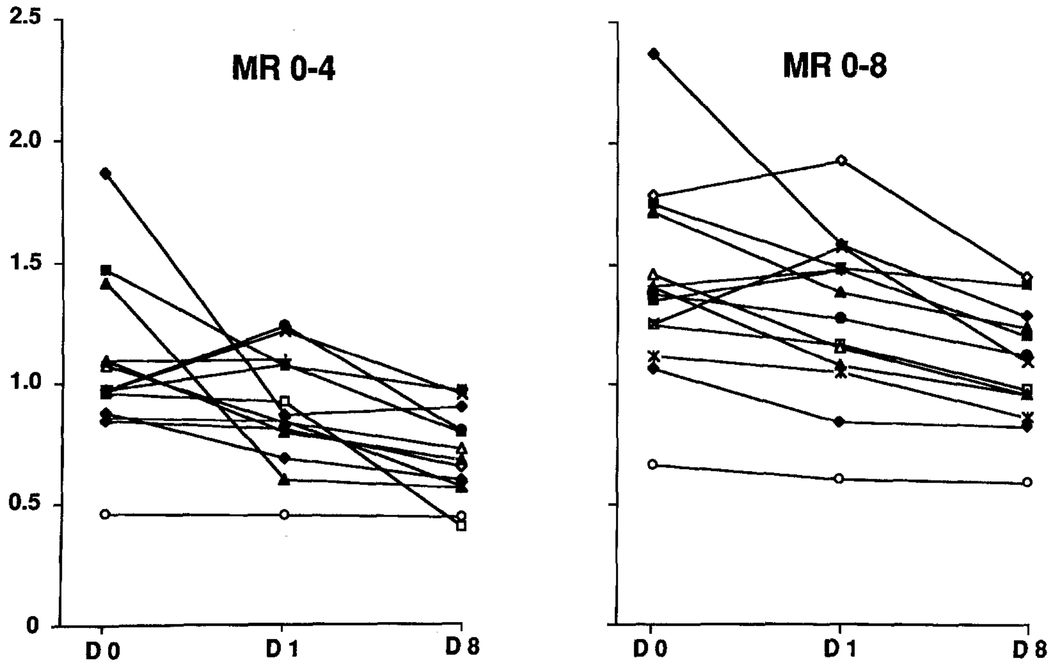

Fig. 3. Urinary diclofenac metabolic ratio (MR; 4′-hydroxydiclofenac/diclofenac) on days 0, 1, and 8.

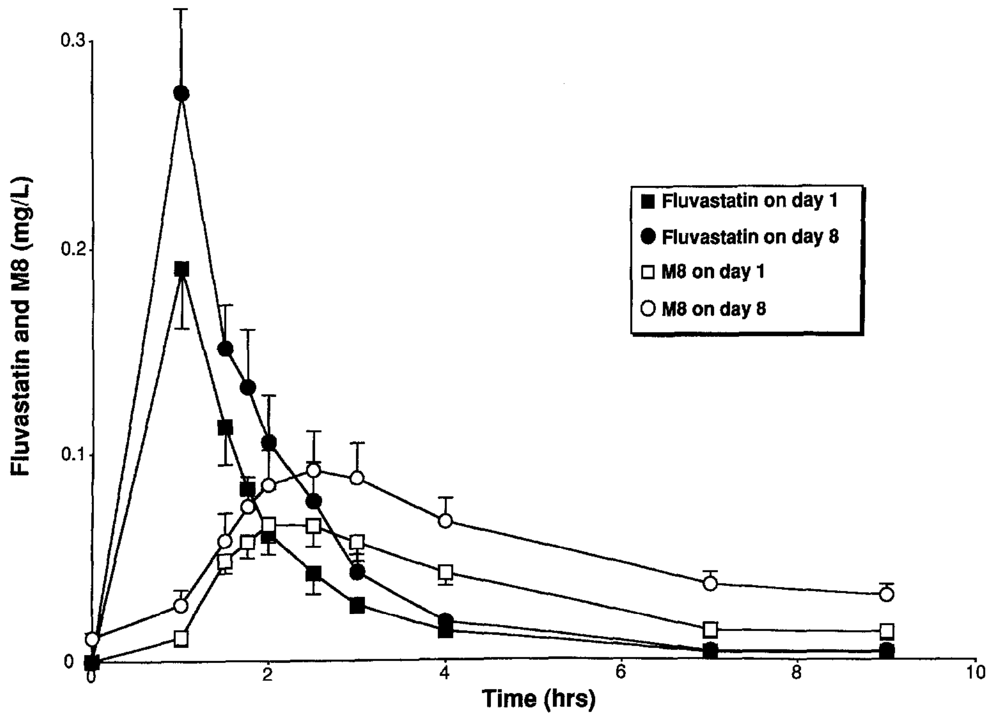

Fig. 4. Mean ± SEM fluvastatin and M8 (the desisopropylpropionic acid derivative of fluvastatin) plasma concentrations on days 1 (start of fluvastatin) and 8 (after 1 week of fluvastatin treatment); $n = 14$.

substrates were unlikely unless mechanisms other than simple competitive inhibition contribute. Accumulation in hepatocytes, most likely by an active transport mechanism (as shown for pravastatin in rat hepatocytes in vitro,[15] is therefore likely to also occur in vivo in humans.

(±)-Fluvastatin, like lovastatin and simvastatin, is therefore likely to interact with P450TB substrates, at least in some individuals.[2-4,16] Significant interactions are expected primarily when fluvastatin is administered simultaneously with high clearance P450TB substrates (e.g., some oral anticoagulants such as acenocoumarol) or when fluvastatin elimination is impaired, leading to higher and sustained inhibiting concentrations. Concomitant administration with substances such as phenytoin,[17] some oral anticoagulants,[7,8] oral antidiabetic agents[16] or nonsteroidad antiinflammatory drugs[6] should therefore be undertaken under careful clinical observation. On the other hand, no clear-cut impact on P450TB-dependent clearance is expected with substrates that are slowly metabolized. Indeed, a pharmacokinetic study after single-dose warfarin administration did not detect any significant interaction.*

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