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The effect of atorvastatin treatment on serum oxysterol concentrations and cytochrome P450 3A4 activity

Janne Hukkanen,[1,2,3,4] Johanna Puurunen,[4,5,6] Tuulia Hyötyläinen,[7] Markku J. Savolainen,[1,2,3,4] Aimo Ruokonen,[8,9] Laure Morin-Papunen,[4,5,6] Matej Orešič,[7] Terhi Piltonen[4,5,6] & Juha S. Tapanainen[4,5,6,10]

[1]Research Center for Internal Medicine, University of Oulu, Oulu, [2]Department of Internal Medicine, Oulu University Hospital, Oulu, [3]Biocenter Oulu, Oulu, [4]Medical Research Center Oulu, Oulu University Hospital and University of Oulu, Oulo, [5]Department of Obstetrics and Gynaecology, Institute of Clinical Medicine, University of Oulu, Oulu, [6]Department of Obstetrics and Gynaecology, Oulu University Hospital, Oulo, Finland, [7]Steno Diabetes Center, Gentofte, Denmark, [8]Department of Clinical Chemistry, Institute of Diagnostics, University of Oulu, Oulu, [9]NordLab Oulu, Oulu University Hospital, Oulu and [10]Department of Obstetrics and Gynaecology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Correspondence
Dr Janne Hukkanen MD, PhD, Department of Internal Medicine, Oulu University Hospital, PO Box 20, FI-90029, Oulu, Finland.
Tel.: +358 8315 6212
Fax: +358 8315 4139
E-mail: janne.hukkanen@oulu.fi

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Atorvastatin is known as a CYP3A4 inhibitor but it is also a PXR-activating inducer of CYP3A4 *in vitro*. The *in vivo* relevance of PXR activation is unknown.
- The cholesterol metabolite 4β-hydroxycholesterol (4βHC) is a convenient marker of CYP3A4 activity but its use has not been evaluated in situations with changing cholesterol concentrations.

WHAT THIS STUDY ADDS

- No induction of CYP3A4 by atorvastatin was detected. Atorvastatin is an inhibitor of CYP3A4 activity.
- The ratio of 4βHC to cholesterol is a feasible index of CYP3A4 activity also when cholesterol concentrations have changed.
- Atorvastatin treatment reduces the concentrations of the oxysterols 4βHC and 5α,6α-epoxycholesterol.

AIMS

Atorvastatin is known to both inhibit and induce the cytochrome P450 3A4 (CYP3A4) enzyme *in vitro*. Some clinical studies indicate that atorvastatin inhibits CYP3A4 but there are no well-controlled longer term studies that could evaluate the inducing effect of atorvastatin. We aimed to determine if atorvastatin induces or inhibits CYP3A4 activity as measured by the 4β-hydroxycholesterol to cholesterol ratio (4βHC : C).

METHODS

In this randomized, double-blind, placebo-controlled 6 month study we evaluated the effects of atorvastatin 20 mg day^{-1} ($n = 15$) and placebo ($n = 14$) on oxysterol concentrations and determined if atorvastatin induces or inhibits CYP3A4 activity as assessed by the 4βHC : C index. The respective 25-hydroxycholesterol and 5α,6α-epoxycholesterol ratios were used as negative controls.

RESULTS

Treatment with atorvastatin decreased 4βHC and 5α,6α-epoxycholesterol concentrations by 40% and 23%, respectively. The mean 4βHC : C ratio decreased by 13% (0.214 ± 0.04 to 0.182 ± 0.04, $P = 0.024$, 95% confidence interval (CI) of the difference –0.0595, –0.00483) in the atorvastatin group while no significant change occurred in the placebo group. The difference in change of 4βHC : C between study arms was statistically significant (atorvastatin –0.032, placebo 0.0055, $P = 0.020$, 95% CI of the difference –0.069, –0.0067). The ratios of 25-hydroxycholesterol and 5α,6α-epoxycholesterol to cholesterol did not change.

CONCLUSIONS

The results establish atorvastatin as an inhibitor of CYP3A4 activity. Furthermore, 4βHC : C is a useful index of CYP3A4 activity, including the conditions with altered cholesterol concentrations.

Introduction

The cholesterol lowering statins (3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors) are among the most widely prescribed medications globally. Statins reduce cardiovascular morbidity and mortality in patients with cardiovascular disease, and more intensive statin therapy confers more benefit than less intensive statin therapy [1]. Drug interactions causing perturbations in statin pharmacokinetics are well-established, the most notorious being the effect of gemfibrozil on cerivastatin blood concentrations resulting in a significant increase of rhabdomyolysis cases [2, 3]. Many other perpetrator drugs are listed in the literature [2, 3]. The data regarding statins as a cause of drug interactions is much more limited although statins are known to inhibit drug metabolizing cytochrome P450 2C9 and 3A4 (CYP3A4) enzymes, and drug transporting multidrug transporter protein 1 (MDR1), breast cancer resistance protein (BCRP), and organic anion-transporting polypeptide 1B1 (OATP1B1) [3, 4]. Atorvastatin is known to inhibit CYP3A4, MDR, BCRP and OATP1B1 [3, 4].

Statins may also induce drug metabolism. Atorvastatin, simvastatin and fluvastatin have been shown in *in vitro* studies to activate the pregnane X receptor (PXR) and, to a much lesser or negligible degree, constitutive androstane receptor (CAR), while pravastatin is not a PXR or CAR agonist [5–9]. Both PXR and CAR have major roles in the induction of drug metabolism and especially in the regulation of CYP3A4 [10]. Rosuvastatin is a weak activator of CAR without PXR affinity [7, 8] and lovastatin is a PXR agonist without CAR affinity [5, 8]. In human hepatocyte cultures, the relative efficacy of statins for CYP induction was atorvastatin > simvastatin > lovastatin > rosuvastatin and no induction was seen with pravastatin [11]. In clinical studies urinary 6β-hydroxycortisol excretion, an established endogenous measure of CYP3A4 activity, was increased with 20 mg day^{-1} simvastatin but not with 20 mg day^{-1} pravastatin administration for 17 days [12], whereas in another study, 10 mg day^{-1} pravastatin for 2 weeks did increase 6β-hydroxycortisol excretion [13]. Rosuvastatin treatment at a dose of 40 mg day^{-1} for 21 days did not result in increased 6β-hydroxycortisol excretion [14]. Four-week 10 mg day^{-1} atorvastatin administration did not affect the urinary 6β-hydroxycortisol : cortisol ratio [15]. Simvastatin reduced the AUC of diltiazem, a CYP3A4 substrate, suggesting induction of CYP3A4 [16]. Thus, there is *in vitro* evidence for the induction of CYP3A4 by statins including atorvastatin but clinical data are limited. Furthermore, as the induction phenomenon takes time to develop fully, the trials with durations of a few weeks are not adequately designed to detect induction.

Serum/plasma 4β-hydroxycholesterol (4βHC), an oxysterol metabolite of cholesterol, is a novel endogenous marker of CYP3A4 and CYP3A5 activity. The concentrations of 4βHC without cholesterol normalization can be used to assess CYP3A activity when cholesterol concentrations are assumed to be stable while the ratio of 4βHC to cholesterol (4βHC : C) is utilized as an index of CYP3A activity when cholesterol concentrations are changing [17]. Another oxysterol, 25-hydroxycholesterol, formed by cholesterol 25-hydroxylase [18], was recently suggested to be an important suppressor of interleukin-1 driven inflammation [19, 20] as well as an antiviral factor [21]. Yet another oxysterol, 5α,6α-epoxycholesterol (5,6-EC), is formed by auto-oxidation of cholesterol and enzymatically in the body but is also present in processed foods [22, 23]. There are no studies examining the effects of statins on 4βHC, 25HC or 5,6-EC concentrations. We recently completed a randomized, double-blind, placebo-controlled 6 month study evaluating the effects of atorvastatin 20 mg day^{-1} on hormonal and metabolic parameters in women with polycystic ovary syndrome (PCOS) [24]. We now set out to analyze the effect of atorvastatin treatment on oxysterol concentrations and, specifically, determine if atorvastatin is an inducer or inhibitor of CYP3A4 activity as assessed by the 4βHC : C ratio while using 25HC : C and 5,6-EC : C ratios as negative controls.

Methods

Subjects and experimental protocol

The protocol of study has been described in detail in a previous publication [24]. Briefly, non-menopausal women with PCOS and reliable non-hormonal contraception, aged 30–50 years, were recruited for the study. Exclusion criteria included type 2 diabetes, medications affecting glucose tolerance, lipid metabolism or steroid synthesis in the preceding 3 months, regular smoking, alcohol abuse, previous ovarian drilling, oophorectomy, or hysterectomy and contraindications for the use of atorvastatin. The subjects were randomized to use either atorvastatin 20 mg day^{-1} ($n = 19$) or placebo ($n = 19$) for 6 months. The study procedures performed were in accordance with the ethical standards of the Helsinki Declaration. Written, informed consent was obtained from each subject. The study was approved by the ethics committee of the Northern Ostrobothnia Hospital District (Oulu, Finland; number 77/2006) and the Finnish Medicines Agency. The trial was registered at ClinicalTrials.gov as NCT01072097 and ClinicalTrialsRegister.eu as 2006-003584-31. Fasting serum samples were collected at baseline and at 3 and 6 months of treatment but for the analyses of oxysterols and desmosterol only the baseline and 6 month samples were used.

Analytical methods and data analyses

Total cholesterol, LDL-C, HDL-C and triglycerides were assayed with an automatic chemical analyzer (Advia 1800; Siemens Healthcare Diagnostics GmbH,

Eschborn, Germany) at the clinical laboratory of Oulu University Hospital (Nordlab, Oulu, Finland) with a method validated for clinical use. Desmosterol, 4βHC, 25HC and 5,6-EC were analyzed with gas chromatography–mass spectrometry (GC-MS) at VTT Technical Research Centre of Finland (Espoo, Finland). The steroid standards were from Steraloids Inc. (Newport, RI, USA).

A 400 μl aliquot of chloroform/methanol (2 : 5, v/v) and 10 μl of internal standard mixture (4βHC-D7, 25HC-D6, desmosterol-D6 and 4(R/S),25-epoxycholesterol-D6; concentration = 0.25 mg l^{-1}) were added to 30 μl of serum samples, and samples were vortexed for 2 min and centrifuged for 5 min at 10 000 rev min^{-1}. Samples were evaporated under nitrogen to dryness and derivatized with 40 μl N-methyl-N-(trimethylsilyl) trifluoroacetamide (60 min, 70°C). Before analysis, 5 μl of injection standard (0.25575 mg l^{-1} of 4,4,-dibromooctafluorobifenyl) was added to the samples. The samples were analyzed with an Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) combined with Agilent 5973 mass selective detector (MSD). The injector (injection volume 1 μl, pulsed splitless injection) and MSD interface temperatures were 280°C, and the oven temperature program was from 50°C to 300°C at a rate of 20°C min^{-1}. The analyses were performed on an Agilent Ultra-2 (5% phenyl methyl siloxane) capillary column (20 m × 0.18 mm internal diameter), film thickness 0.1 μm). Six point calibration was done for each target analyte using labelled internal standards for normalization (concentration = 0.1–2 μg ml^{-1}). The method proved to be linear at this concentration range. Control serum samples and blank samples were analyzed together with the samples. The samples were analyzed in duplicate. Relative standard deviation (precision) was 8.0% for 4βHC, 1.5% for 25HC, 1.6% for 5,6-EC and 6.0% for desmosterol. The accuracy (% of expected value) over a range of concentrations varied from 100 to 110%, 99 to 125%, 86 to 115% and 94 to 99% for 4βHC, 25HC, 5,6-EC and desmosterol, respectively. The lower limit of quantification was 3.6 ng ml^{-1} for 4βHC, 3.5 ng ml^{-1} for 25HC, 4.0 ng ml^{-1} for 5,6-EC and 17.5 ng ml^{-1} for desmosterol.

The molar ratio of 4βHC to cholesterol × 10 000 was used as the indicator of CYP3A4 activity, and the change in 4βHC : C during treatment was used as the parameter to test if atorvastatin affected CYP3A4 activity in comparison with placebo. The Mann–Whitney *U*-test was used to analyze differences in oxysterol : cholesterol ratios between the study groups. Student's *t*-test was used to analyze the intra-individual changes during treatments. Pearson correlation coefficients were calculated to analyze correlations between cholesterol and oxysterols. $P < 0.05$ was considered statistically significant.

Results

The subjects were randomized to use either atorvastatin 20 mg day^{-1} ($n = 19$) or placebo ($n = 19$). After allocation, one subject from the placebo group was excluded because of menorrhagia, and three subjects (one subject in the atorvastatin group and two in the placebo group) were excluded as type 2 diabetes was diagnosed based on baseline OGTT. During the study, four women (two subjects in the atorvastatin group and two in the placebo group) were excluded due to non-adherence to the study protocol, one subject from the placebo group withdrew because of myalgia and one woman from the atorvastatin group withdrew because of arthralgia. In the placebo group, one patient dropped out between the 3 month and 6 month visits and the 3 month serum sample was used for the oxysterol and cholesterol analyses. Thus, serum samples from 15 women in the atorvastatin group and 14 women in the placebo group were available for the analyses. The mean age of the subjects in the atorvastatin group was 40 years (SD ± 5.9; range 29–47), the mean weight 80 kg (SD ± 21.5; range 53–134) and the body mass index (BMI) 30.4 (SD ± 8.6; range 19.9–53.8). In the placebo group, the mean age was 38 years (SD ± 4.6; range 30–46), the mean weight 76 kg (SD ± 18.2; range 56–116) and the BMI 28.3 (SD ± 7.3; range 20.2–48.1). All the subjects were Caucasian. The concentrations of atorvastatin were not monitored but as cholesterol and LDL-C concentrations were reduced by a minimum of 20% (mean 31%) and 32% (mean 46%), respectively, it can be concluded that all the subjects were adherent in the atorvastatin arm.

Four subjects in the atorvastatin group and two in the placebo group with oligomenorrhoea used progestin (dydrogesterone 10 mg for 10 days) to induce menstrual bleeding before the examinations at baseline and at 3 and 6 months. In the placebo group, one woman used thyroxine for hypothyroidism and one subject inhaled budesonide and salbutamol for asthma. In the atorvastatin group, one subject used fluticasone and one woman salbutamol for asthma. Additionally, three women in the atorvastatin group had hypertension (treated with ramipril, hydrochlorothiazide and the losartan-hydrochlorothiazide-amiloride combination, respectively).

Atorvastatin treatment decreased 4βHC concentration (44.4 ng ml^{-1} to 26.4 ng ml^{-1}, a mean 40% decrease, $P < 0.001$) and 4βHC normalized with cholesterol (the mean 4βHC : C, 0.214 ± 0.04 to 0.182 ± 0.04, a mean 13% decrease, $P = 0.024$, 95% confidence interval (CI) of the difference −0.0595, −0.00483) while 4βHC and 4βHC : C did not change in the placebo group (Table 1). The difference in change of 4βHC : C between study arms was statistically significant ($P = 0.020$, 95% CI of the difference −0.069, −0.0067). Also the difference in percentage change in 4βHC : C between the treatments was statistically significant ($P = 0.033$).

Table 1

The effect of atorvastatin and placebo on 4β-hydroxycholesterol (4βHC), 25-hydroxycholesterol (25HC), 5α,6α-epoxycholesterol (5,6-EC) and desmosterol, and their respective ratios to total cholesterol (C) on women with polycystic ovary syndrome

	Atorvastatin 20 mg (n = 15)			Placebo (n = 14)			Between groups P value
	Baseline	6 months	Change (P value)	Baseline	6 months	Change (P value)	
4βHC : C	0.21 ± 0.04	0.18 ± 0.04	−0.032 ± 0.05 (0.024)	0.20 ± 0.04	0.21 ± 0.05	0.0055 ± 0.03 (NS)	0.020
4βHC (ng ml^{-1})	44.4 ± 5.7	26.4 ± 6.9	−18.0 ± 7.8 (<0.001)	41.2 ± 8.3	41.0 ± 9.2	−0.14 ± 7.2 (NS)	<0.001
Cholesterol (mmol l^{-1})	5.2 ± 0.8	3.6 ± 0.6	−1.6 ± 0.5 (<0.001)	5.1 ± 1.1	5.0 ± 1.0	−0.12 ± 0.6 (NS)	<0.001
25HC : C	0.040 ± 0.01	0.046 ± 0.02	0.0057 ± 0.018 (NS)	0.044 ± 0.01	0.040 ± 0.01	−0.0044 ± 0.012 (NS)	0.25
25HC (ng ml^{-1})	8.4 ± 2.3	6.6 ± 2.3	−1.8 ± 2.8 (0.026)	8.6 ± 1.6	7.7 ± 1.4	−0.94 ± 1.8 (0.069)	0.38
5,6-EC : C	1.21 ± 0.40	1.28 ± 0.34	0.070 ± 0.53 (NS)	1.23 ± 0.26	1.35 ± 0.32	0.12 ± 0.36 (NS)	1.0
5,6-EC (ng ml^{-1})	261 ± 127	188 ± 60	−73 ± 118 (0.030)	255 ± 86	269 ± 81	14 ± 81 (NS)	0.018
Desmosterol : C	2.10 ± 0.48	1.96 ± 0.37	−0.14 ± 0.55 (NS)	1.98 ± 0.36	2.16 ± 0.39	0.18 ± 0.53 (NS)	0.15
Desmosterol (ng ml^{-1})	427 ± 138	270 ± 61	−156 ± 119 (<0.001)	384 ± 97	407 ± 92	23 ± 107 (NS)	<0.001

Data are presented as mean ± SD. The oxysterol to cholesterol ratios are presented as molar ratios × 10 000. The Mann–Whitney U-test was used to analyze differences in the ratios between the study groups. The paired Student's t-test was used to analyze the intra-individual changes during treatments. NS, not statistically significant.

The mean 25HC concentrations decreased during the atorvastatin arm but the change was not significant when compared with the placebo arm (Table 1). The mean 5,6-EC concentration was reduced by atorvastatin and this change was significant also when compared with the placebo arm. Additionally, we measured desmosterol, a marker of cholesterol synthesis. Desmosterol concentrations were reduced by atorvastatin and the change was significant when compared with placebo. Although 25HC, 5,6-EC and desmosterol were affected by atorvastatin to a varying degree, their concentrations corrected by total cholesterol were not affected (Table 1). At baseline, serum cholesterol concentrations correlated significantly with 4βHC, 5,6-EC and desmosterol but not with 25HC levels (Table 2). The serum concentrations of 5,6-EC correlated significantly with those of 4βHC and desmosterol.

Discussion

This study demonstrates that atorvastatin treatment inhibits CYP3A4 as measured by 4βHC : C, a well-established index of CYP3A4 activity. This is the first time that this index has been used to assess the interaction potential of any statin. As the ratios of oxysterols 25HC and 5,6-EC to total cholesterol were not affected by statin treatment, it is highly unlikely that the statin effect on 4βHC : C could be attributable to the reduced cholesterol concentration. Thus, the mean 40% reduction in serum 4βHC is caused by both the statin effect on cholesterol concentration via inhibition of HMG-CoA reductase and the inhibitory effect of atorvastatin on CYP3A4-mediated 4βHC formation. In contrast, the mean 13% decrease in 4βHC : C reflects the inhibition of CYP3A4 by atorvastatin and not the reduced cholesterol concentration. Therefore, we propose 4βHC : C to be a valuable measure of CYP3A4 activity also when cholesterol concentrations are drastically changing, as happens during statin therapy. Since 4βHC has a long half-life (~17 days) [17], it should be ensured that both cholesterol and 4βHC concentration have reached a new steady-state after the introduction of a test compound. Thus the 4βHC : C index is not suitable for situations with rapidly fluctuating cholesterol concentrations.

Atorvastatin is a CYP3A4 inhibitor based on *in vitro* experiments but pharmacokinetic and clinical studies with CYP3A4-metabolized probe drugs are to some extent contradictory. In atorvastatin-treated patients *vs.* controls the clearance of intravenous midazolam was reduced by atorvastatin [25]. In contrast, in a placebo-controlled crossover study, 10 mg atorvastatin for 2 weeks did not affect the pharmacokinetics of oral midazolam [26]. A parallel arm study in atorvastatin-treated patients and controls did not detect an inhibitory effect of atorvastatin on intravenous midazolam pharmacokinetics [27]. The area under the curve (AUC) of verapamil was increased and the ratio of norverpamil : verapamil AUCs was decreased with concurrent 40 mg atorvastatin dosing indicating the inhibition of both MDR1 and CYP3A4 [28]. Atorvastatin 80 mg co-administration for

Table 2

The Pearson correlation coefficients of total cholesterol and oxysterols at the baseline (n = 29)

	Cholesterol	4βHC	25HC	5,6-EC
4βHC	0.462 (P = 0.012)	–		
25HC	0.053 (NS)	−0.141 (NS)	–	
5,6-EC	0.704 (P < 0.001)	0.441 (P = 0.017)	−0.049 (NS)	–
Desmosterol	0.657 (P < 0.001)	0.172 (NS)	0.256 (NS)	0.705 (P < 0.001)

4βHC 4β-hydroxycholesterol; 25HC 25-hydroxycholesterol; 5,6-EC 5α,6α-epoxycholesterol; NS not statistically significant.

7 days increased terfenadine AUC [29]. The AUC of norethisterone and ethinylestradiol were increased with 22 day administration of 40 mg atorvastatin (Lipitor Summary of Product Characteristics). Four week 10 mg day^{-1} atorvastatin administration did not affect 6β-hydroxycortisol excretion [15]. Atorvastatin inhibits CYP3A4-mediated activation of clopidogrel but there is no consistent evidence of harm for patients with cardiovascular disease using the combination of atorvastatin and clopidogrel (reviewed by Bates *et al.* [30]). However, in a population-based pharmacoepidemiological study with patients on clopidogrel treatment, thrombosis complications leading to hospitalizations were more prevalent and the hospitalizations due to bleedings less prevalent in the atorvastatin group when compared with the control group [31]. Our results strengthen the conclusion that CYP3A4 is inhibited by atorvastatin treatment, potentially leading to drug–drug interactions. The magnitude of inhibition at a dose of 20 mg day^{-1} was relatively small. In higher atorvastatin doses inhibition is likely to be more pronounced. The clinical consequences of CYP3A4 inhibition by atorvastatin are determined by the properties of the concomitant medications such as the width of the therapeutic range and the possible alternative metabolic pathways, the temporal relationship between administration of atorvastatin and the affected drug, and the individual propensity to adverse effects.

Although there are *in vitro* data suggesting that atorvastatin is as an inducer of CYP enzymes via PXR activation, there are no previous randomized longer term studies that could evaluate the inducing effect of atorvastatin on CYP3A4 activity. The results of our randomized clinical study show that the induction of CYP3A4 *in vivo* is unlikely. The 6 month duration of the study would have given enough time for the induction to occur. It is known that the induction phenomenon takes weeks to months to fully develop [32]. Nevertheless, the induction of CYP3A4 masked by the inhibition of CYP3A4 cannot be absolutely ruled out.

Several subjects used concomitant medications during the study. None of these was known to inhibit CYP3A4. Six patients used dydrogesterone before the study visits to induce menstruation. Although intramuscular (but not oral) medroxyprogesterone acetate treatment has been shown to induce CYP3A4-mediated metabolism [33], there is no information on the CYP-inducing effects of dydrogesterone. The concentration of 4βHC and the 4βHC : C ratio did not differ at baseline between the users and non-users of dydrogesterone (data not shown). Thus, dydrogesterone is unlikely to induce CYP3A4 and affect the results of this study. Furthermore, as PCOS is not known to affect CYP3A4 activity, the results should be generalizable to other patient populations.

This study presents important new biological information on the oxysterols 25HC and 5,6-EC. Recently, there has been much interest on 25HC since it was shown to be an antiviral factor and suppressor of interleukin-1β driven inflammation [19–21]. Thus, it is reassuring that atorvastatin treatment did not significantly affect circulating 25HC concentration. It was recently suggested that 25HC might be partly formed by CYP3A4 [34]. Our findings that the 25HC : C ratio was not affected by the CYP3A4 inhibitor atorvastatin and that 4βHC and 25HC concentration did not correlate at baseline (Table 2), indicate that CYP3A4 is not a significant factor in the formation of 25HC. The oxysterol 5,6-EC is produced enzymatically (peroxidases, lipoxygenases, and monoxygenases) and by autooxidation of cholesterol but it is also present in foods such as eggs, meat, and cheese [22, 23]. Atorvastatin treatment decreased 5,6-EC concentration and it was highly correlated with the concentration of desmosterol, a marker of cholesterol synthesis. This suggests that the endogenous formation is a more important source of 5,6-EC than diet.

In conclusion, we established atorvastatin as an inhibitor of CYP3A4 enzyme as suggested by some, but not all, previous studies, and indicated that atorvastatin is not an inducer of CYP3A4 *in vivo* as suggested by *in vitro* studies. Importantly, we propose 4βHC : C as a useful index of CYP3A4 activity when cholesterol concentrations have changed. Furthermore, we provide evidence that CYP3A4 activity and cholesterol concentration are not controlling the formation of 25HC whereas 5,6-EC concentrations are linked with endogenous cholesterol synthesis.

Competing Interests

Atorvastatin (Lipitor®) and placebo were provided by Pfizer, Inc. (New York, NY, USA), which did not have any input into the study design, its execution or interpretation of the findings. The authors have nothing else to disclose. All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

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Authors' Contributions

JP was the principal investigator. JH wrote the initial version of the manuscript, and all authors critically commented, revised, and gave the final approval to the manuscript, AR, JH, JP, TP, MJS, LM-P and JST designed the study, JP, TP, LM-P and JST performed the research, TH and MO performed the oxysterol analyses and JH analyzed the data.

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