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ARTICLE

Systemic Lupus Erythematosus Activity Affects the Sinusoidal Uptake Transporter OATP1B1 Evaluated by the Pharmacokinetics of Atorvastatin

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The present study assessed the effect of systemic lupus erythematosus (SLE) activity, a chronic and inflammatory autoimmune disease, on the sinusoidal uptake transporter OATP1B1 using atorvastatin (ATV) as a probe drug. Fifteen healthy subjects, 13 patients with controlled SLE (SLEDAI 0–4), and 12 patients with uncontrolled SLE (SLEDAI from 6 to 15), all women, were investigated. Apparent total clearance of midazolam (MDZ), a marker of CYP3A4 activity, did not vary among the three investigated groups. The controlled and uncontrolled SLE groups showed higher plasma concentrations of MCP-1 and TNF-α, while the uncontrolled SLE group also showed higher plasma concentrations of IL-10. The uncontrolled SLE group showed higher area under the curve (AUC) for ATV (60.47 (43.76–83.56) vs. 30.56 (22.69–41.15) ng hour/mL) and its inactive metabolite ATV-lactone (98.74 (74.31–131.20) vs. 49.21 (34.89–69.42) ng·hour/mL), and lower apparent total clearance (330.7 (239.30–457.00) vs. 654.5 (486.00–881.4) L/hour) and apparent volume of distribution (2,609 (1,607–4,234) vs. 7,159 (4,904–10,450) L), when compared to the healthy subjects group (geometric mean and 95% confidence interval). The pharmacokinetics of ATV and its metabolites did not differ between the healthy subject group and the patients with controlled SLE group. In conclusion, uncontrolled SLE increased the systemic exposure to both ATV and ATV-lactone, inferring inhibition of OATP1B1 activity, once *in vivo* CYP3A4 activity assessed by oral clearance of MDZ was unaltered. The inflammatory state, not the disease itself, was responsible for the changes described in the uncontrolled SLE group as a consequence of inhibition of OATP1B1, because systemic exposure to ATV and its metabolites were not altered in patients with controlled SLE.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Studies have reported that increased plasma concentrations of pro-inflammatory cytokines modulate the activity of CYP enzymes as well as efflux and/or uptake transporters, including OATP1B1. Systemic lupus erythematosus (SLE) is a chronic inflammatory disease, and sinusoidal uptake transporter OATP1B1 is responsible for distribution of atorvastatin (ATV) to the liver.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ We hypothesized that the high concentrations of cytokines observed in patients diagnosed with uncontrolled SLE could reduce the activity of OATP1B1 and limit the distribution of ATV to the liver.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ Uncontrolled SLE with high plasma concentrations of IL-10, MCP-1, and TNF-α increases the systemic exposure and apparent volume of distribution and decreases the total apparent clearance of ATV, inferring inhibition of OATP1B1 activity.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ Inflammation, not SLE, is responsible for the changes in the ATV pharmacokinetics observed in uncontrolled SLE because its systemic exposure was unchanged in controlled SLE. Thus, the progression of the disease is clinically relevant for dose adjustments of OATP1B1 substrates.

In vitro, preclinical, and clinical studies indicate that diseases associated with inflammation and high concentrations of pro-inflammatory cytokines can result in downregulation of metabolizing enzymes, and downregulation or upregulation of membrane transporters, as well as changes in the body fluid volume (edema) and the plasma protein concentrations (decrease of albumin and increase of alpha-1-acid glycoprotein levels). Thus, inflammation may reduce clearance and

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increase plasma drug concentration via metabolism and/or membrane transporters.[1–3]

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by high levels of auto-antibodies and immune complex deposition. Cytokines, such as interleukin-1 (IL-1), IL-6, IL-10, tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), among others, play an essential role in SLE pathogenesis, and their balance determines disease activity.[4–6] Approximately 30% of patients with SLE present dyslipidemia characterized by decreased high-density lipoproteins and increased total cholesterol, low-density lipoproteins (LDLs), very-low-density lipoproteins, triglycerides, and apolipoprotein B. After 3 years from the diagnosis of the disease, dyslipidemia can affect up to 60% of the patients with SLE.[7–11]

The efficacy of statins used to treat hypercholesterolemia depends on the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase in the liver, to where the drug is distributed through the sinusoidal influx transporter OATP1B1.[12–14]

Atorvastatin (ATV) calcium is administered in its active, highly soluble, and permeable acid form (log $P_{octanol:water}$ 4.07), and it is subject to presystemic elimination in both gut and liver with oral bioavailability of ~ 14%.[15–17] ATV is interconverted to its inactive metabolite ATV lactone (ATV-LAC) by both the CoASH-dependent and the acyl glucuronide intermediate pathways. ATV-LAC is hydrolyzed to its ATV acid form chemically or enzymatically by esterase and paraoxonases.[18] The acid and lactone forms of ATV are metabolized by CYP3A4 to the corresponding hydroxylated para-metabolites and ortho-metabolites (*p*-OH-ATV and *o*-OH-ATV). The active metabolites *p*-OH-ATV and *o*-OH-ATV acid are responsible for 70% of the total inhibitory activity of plasma 3-hydroxy-3-methylglutaryl coenzyme A reductase.[19,20] Although lactone forms of ATV are inactive regarding the hypolipidemic effects, high plasma concentrations of these metabolites are associated with myopathy. Thus, the ratios of lactone metabolite/unchanged drug plasma concentrations are biomarkers for ATV-associated myopathy.[21]

The sinusoidal uptake transporter OATP1B1 is responsible for the transport of both ATV and ATV acid metabolites alike. In contrast, the distribution of the lactone and its hydroxylated metabolites dependent on OATP1B1 is limited, probably because passive diffusion is more critical for lipophilic lactones when compared with the more hydrophilic acid forms. ATV and its acid metabolites and lactones are actively excreted into the bile via the MDR1 and the BCRP transporters.[15,19,22,23]

Following a single intravenous dose of 5 mg ATV in healthy subjects, its total clearance is 625 mL/minute, the hepatic extraction ratio is 0.42, and the volume of distribution is 381 L. The protein binding of ATV is higher than 90%. The elimination half-life of ATV is 7 hours, whereas higher values (13–16 hours) are reported for its active acid metabolites. Renal excretion plays a minor role (< 1%) in the elimination of ATV and its metabolites.[17,19]

The instantaneous equilibrium between the unbound drug concentrations in the blood and the liver occurs only for lipophilic drugs that do not depend on sinusoidal membrane transporters. ATV is an example of a drug in which metabolism and/or biliary excretion are much faster processes than the sinusoidal influx transport (i.e., its hepatic clearance depends on the sinusoidal influx transport because metabolic clearance (CL_m) plus canalicular efflux clearance (CL^c_{ef}) is much higher than the sinusoidal efflux clearance). Thus, hepatic clearance of ATV is dependent on the activity of sinusoidal membrane OATPs, and not on CL_m. Previous studies show that inhibition of OATPs results in a 12-fold increase in ATV area under the curve (AUC) values, whereas inhibition of CYP3A4 does not affect ATV plasma concentrations. However, CYP3A4 inhibition increases ATV liver exposure and, consequently, alters the pharmacological response, whereas OATP inhibition increases systemic exposure, but does not affect liver exposure.[24]

The pro-inflammatory cytokines TNF-α or IL-6 downregulate mRNA levels of OATP1B1, OATP1B3, and OATP2B1, among other canalicular drug transporters, in primary human hepatocytes. TNF-α and IL-6 concomitantly reduce OATP1B1 protein expression and transport activity in human hepatocytes in a dose-dependent manner. Cellular and molecular mechanisms responsible for the pro-inflammatory cytokine-related regulation of transporters in human primary hepatocytes remain to be determined, although transcriptional and post-transcriptional mechanisms probably may be involved.[25]

Considering that the high plasma concentrations of pro-inflammatory cytokines observed in patients diagnosed with SLE could reduce the activity of both influx transporters, such as OATP1B1, and enzymes involved in the drug metabolism process, such as CYP3A4, the present study aims to assess the effect of controlled and uncontrolled SLE on the activity of the sinusoidal influx transporter OATP1B1, evaluated by the pharmacokinetics of ATV and its five metabolites.

CLINICAL STUDY AND METHODS

Clinical protocol

The research protocol was approved by the Research Ethics Committees of the local hospital and of the School of Pharmaceutical Sciences of Ribeirao Preto, University of Sao Paulo, Brazil. The research protocol was registered on the Brazilian Registry of Clinical Trials (ReBEC, www.ensaiosclinicos.gov.br) under the ID number RBR-6p37zr.

Female patients with SLE were diagnosed according to the American College of Rheumatology standards, considering the presence of at least four criteria, either serially or simultaneously, during any interval of clinical observation. Patients with SLE were classified according to disease activity (Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)) with scores of 0–105, which includes clinical and laboratory tests (complete blood count, urinalysis, serum complement dosage, and serum titer of anti-DNA antibody).[26,27]

Patients with moderate (SLEDAI 6–10) to high disease activity (SLEDAI 11–19) presenting clinical and laboratory evidence of inflammation were included in the uncontrolled SLE group. Patients with none (SLEDAI 0) to mild disease activity (SLEDAI 1–5), no active kidney disease, and no clinical or laboratory evidence of inflammation[27–29] were included in the controlled SLE group. The healthy subjects were enrolled matched in terms of sex, age, and weight to uncontrolled and controlled SLE groups.

At the time of blood sampling, all included patients with SLE were on stable doses of oral prednisone (5–40 mg/day), chloroquine (250 mg/day), or hydroxychloroquine (400 mg/day), and immunosuppressant (azathioprine, mycophenolate mofetil, or cyclophosphamide), none of which are inhibitors of the OATP1B1 transporter.[30] Other concomitant treatments included acetylsalicylic acid, amlodipine, atenolol, calcium carbonate, clonidine, oral contraceptives, enalapril, escitalopram, folic acid, furosemide, hydralazine, hydrochlorothiazide, losartan, ranitidine, vitamin D, and warfarin.

The sample size was calculated with the aid of Power and Sample Calculation software version 3.0.43,[31,32] using the area under the plasma concentration vs. time curve data of ATV (AUC mean ± SD: 64.0 ± 21.3 ng·hour/mL) obtained in the investigation of healthy subjects who received a single dose of 40 mg of ATV.[33] The inclusion of at least 12 participants in each group allows for the observation of differences of at least 40% in ATV AUC values among the investigated groups, with a significance level of 5% and test power of 80%.

The participants were included in the study after signing the prior informed consent form and were then allocated into the healthy subjects group (n = 15, 18–50-years-old, body mass index (BMI) range of 21.28–30.50 kg/m^2), controlled SLE group (n = 13, SLEDAI 0–4, 20–55 years-old, BMI range of 18.67–32.69 kg/m^2), and uncontrolled SLE group (n = 12, SLEDAI 6–15, 22–57 years-old, BMI range of 21.48–34.81 kg/m^2; **Table 1**). The participants were admitted to the Clinical Research Center of the General Hospital of the Ribeirao Preto Medical School, University of Sao Paulo.

All subjects underwent clinical and laboratory tests for liver (alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltransferase, total bilirubin and fractions, and alkaline phosphatase), renal (urea and creatinine), and metabolic evaluation (total cholesterol, triglycerides, high-density lipoprotein, LDL, and fasting blood glucose), total serum protein levels, albumin, alpha-1-acid glycoprotein, and total blood count. Subsequently, 2 mL of blood was collected into an EDTA tube for the assessment of plasma cytokines.

On the first day of admission (D1), the participants were evaluated for *in vivo* CYP3A4 activity. They received a single oral dose of 7.5 mg of midazolam (MDZ; Dormonid, Roche), and serial blood samples of 4 mL each were collected into heparin tubes before and at times 0.25, 0.5, 1, 2, 3, 4, and 6 hours after drug administration. The blood samples were centrifuged, and plasma was separated and kept at –80°C until the analysis.

On the second day of the study (D2), the participants from each group were randomized to a single dose of 20, 40, or 80 mg of film-coated tablets of ATV calcium (Lipitor; Pfizer, New York, NY). Serial blood samples of 5 mL each were collected into sodium fluoride tubes (esterase inhibitor used to stabilize ATV and its metabolites during storage)[20] immediately before and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 22, 24, 30, and 36 hours after ATV administration. Plasma samples were stored at –80°C until analysis.

Analytical methods

The analysis of the total plasma concentration of ATV and its five metabolites (*o*-OH-ATV, *p*-OH-ATV, ATV-lactone, *o*-OH-ATV-lactone, and *p*-OH-ATV-lactone) and the unbound plasma concentration of ATV were performed by ultraperformance liquid chromatography-tandem mass spectrometry,[34] according to a previous method developed and validated by our research group. The method of analysis of the total plasma concentration of ATV and its 5 metabolites was linear in the range of 25–200 ng/mL plasma. The

Table 1 Anthropometric data and CL/F of MDZ for healthy subjects (n = 15), patients with controlled SLE (n = 13), and patients with uncontrolled SLE (n = 12)

	Healthy subjects			Controlled SLE				Uncontrolled SLE			
Participants	Age, years	BMI, kg/m^2	CL/F$_{MDZ}$, mL/minute/Kg	Age, years	BMI, kg/m^2	CL/F$_{MDZ}$, mL/minute/kg	SLEDAI	Age, years	BMI, kg/m^2	CL/F$_{MDZ}$, mL/minute/kg	SLEDAI
1	22	27.99	26.22	38	30.67	23.59	1	30	23.24	21.71	8
2	42	26.60	14.97	21	22.43	19.68	4	48	34.81	18.13	6
3	32	27.60	25.97	34	32.00	56.61	0	22	25.26	44.53	15
4	49	30.50	39.85	55	32.69	27.99	4	35	31.05	20.67	6
5	33	28.29	24.55	28	28.62	12.04	4	47	26.85	—	9
6	21	24.43	46.60	39	32.07	24.96	0	57	32.38	42.37	6
7	25	23.56	26.92	37	21.02	31.39	0	38	21.48	46.05	10
8	35	21.46	42.84	20	28.87	19.85	0	23	27.27	49.16	6
9	28	21.86	47.78	29	32.11	33.91	0	34	30.90	29.51	12
10	50	22.62	45.10	33	18.67	49.13	2	37	26.51	66.02	8
11	33	29.28	29.23	53	26.58	23.33	0	43	21.66	44.62	12
12	18	29.00	60.44	50	24.14	50.49	2	29	25.18	61.03	10
13	36	21.28	31.46	34	25.62	19.49	2				
14	26	29.98	34.60								
15	43	22.31	42.02								

SLEDAI scores are presented for patients with both controlled and uncontrolled SLE. Data presented as individual values.
—, not collected; BMI, body mass index; CL/F$_{MDZ}$: apparent total clearance of midazolam;. SLE, systemic lupus erythematosus.

method of analysis of the unbound plasma concentration of ATV was linear in the range of 6.25 pg–25 ng/mL plasma ultrafiltrate. The analysis of MDZ in plasma was performed by liquid chromatography-tandem mass spectrometry[35] in the concentration range of 0.1–100 ng/mL plasma. The coefficients of variation and the relative standard errors of the accuracy and precision analyses were lower than 15% for both cited methods.

Plasma cytokines IFN-γ, IL-6, TNF-α, IL-10, IL-1β, IL-8, and MCP-1[4–6,36] were quantified by flow cytometric assay, using Milliplex MAP Human Cytokine/Chemokine Kit (Magnetic Bead Panel Immunoassay; Luminex Corporation, Austin, TX).

Pharmacokinetics analysis

The *in vivo* CYP3A4 activity and the pharmacokinetic parameters of ATV and its metabolites were calculated based on total plasma concentrations vs. time curves using Phoenix WinNonlin version 6.4 (Certara USA, Princeton, NJ). Monocompartmental, bicompartmental, and noncompartmental models were used for MDZ, ATV, and ATV metabolites, respectively.

The unbound plasma concentration of ATV was assessed at the time of observation of the maximum total plasma concentration (C_{max}) of ATV. The unbound fraction of ATV in plasma was determined based on the ratio of unbound plasma concentration/total plasma concentration.

Statistical analysis

Statistical analysis was performed with R[37] software to obtain the parameter's geometric means and 95% confidence intervals, and the groups were compared by analysis of variance (ANOVA) test with Tukey's Honestly Significant Difference, with a significance level of 5%. The figures were generated with the ggplot2 package.[38]

RESULTS

The anthropometric data and the apparent total clearance values of MDZ for three investigated groups are presented in **Table 1** as individual data. Plasma concentrations of IFN-γ, IL-6, TNF-α, IL-10, IL-1β, IL-8, and MCP-1 cytokines are presented in **Table 2** for the three investigated groups. Plasma concentrations of IL-10, MCP-1, and TNF-α were higher ($P < 0.05$) in the uncontrolled SLE group when compared with the healthy subjects group (**Table 2**). Differences from the controlled SLE group were observed for MCP-1 and TNF-α when compared with the healthy subjects group. No difference in cytokine levels between controlled SLE and uncontrolled SLE was found in the present study.

The plasma concentration vs. time curves (0–36 hours) of ATV and its 5 metabolites were normalized to a 20 mg dose of ATV, and the data are presented in **Figure 1**. The C_{max}/dose and AUC/dose ratios showed linearity in all investigated groups following a single oral dose of ATV (20, 40, or 80 mg) administered as film-coated tablets of ATV calcium; **Figure S1**.

The pharmacokinetic parameters of unchanged ATV are presented in **Table 3**, with significant differences ($P < 0.05$) observed only between uncontrolled SLE and the healthy subjects groups. Data show higher AUC and C_{max} values and lower apparent total clearance values and apparent volume of distribution (**Figure 2**) for patients in the uncontrolled SLE group when compared with the healthy subjects group.

The correlation ($P = 0.01088$) between MCP-1 plasma concentrations and apparent total clearance of ATV values is shown in **Figure 3** for all investigated participants ($n = 40$).

Table 4 presents the pharmacokinetic parameters of ATV metabolites (normalized to ATV 20 mg dose) and the AUC metabolic ratios of *o*-OH-ATV/ATV, ATV-LAC/ATV, *o*-OH-ATV-LAC/ATV, *p*-OH-ATV/ATV, and *p*-OH-ATV-LAC/ATV for the three groups, with significant differences ($P < 0.05$) observed only for the ATV-LAC metabolite. Data show higher ATV-LAC $AUC_{0–36\ hours}$ and C_{max} values for the uncontrolled SLE group when compared to the healthy subjects group.

DISCUSSION

The present study aims to evaluate the impact of controlled and uncontrolled SLE on the activity of the sinusoidal uptake transporter OATP1B1 using ATV as a probe drug. Fifteen healthy subjects, 13 patients with controlled SLE (SLEDAI 0–4), and 12 patients with uncontrolled SLE (SLEDAI 6–15), all women, were investigated. SLEDAI scores for patients with controlled SLE and uncontrolled SLE are presented individually in **Table 1**.

All healthy subjects and patients with controlled SLE presented biochemical tests within the reference ranges (data not shown). However, some patients with uncontrolled SLE had increased plasma concentrations of

Table 2 Cytokine plasma concentrations for healthy subjects ($n = 15$), patients with controlled SLE ($n = 13$) and uncontrolled SLE ($n = 12$)

Cytokine, pg/mL	Healthy subjects	Controlled SLE	Uncontrolled SLE
IFN-γ	17.51 (12.90–23.77)	26.53 (21.57–32.64)	20.75 (13.67–31.50)
IL-10	29.25 (22.40–38.21)	47.87 (38.57–59.40)	41.34△ (27.86–61.35)
IL-1β	5.44 (3.92–7.56)	8.16 (6.45–10.31)	4.96 (2.83–8.68)
IL-6	6.68 (4.00–11.14)	10.40 (7.39–14.64)	7.72 (5.02–11.87)
IL-8	3.56 (2.24–5.66)	5.71 (4.17–7.81)	7.68 (5.12–11.54)
MCP-1	231.00 (198.46–268.88)	389.31 (311.00–487.35)	468.07△ (354.17–618.61)
TNF-α	21.37 (16.34–27.95)	36.09 (27.92–46.64)	35.61△ (25.16–50.42)

Data presented as geometric means and 95% confidence interval
IFN-γ, gamma interferon; IL, interleukin; MCP-1, monocyte chemotactic protein-1; SLE, systemic lupus erythematosus; TNF-α, tumor necrosis factor alpha.
$P < 0.05$ analysis of variance: (healthy subjects vs. controlled SLE), △(healthy subjects vs. uncontrolled SLE), no differences were observed between controlled SLE vs. uncontrolled SLE.

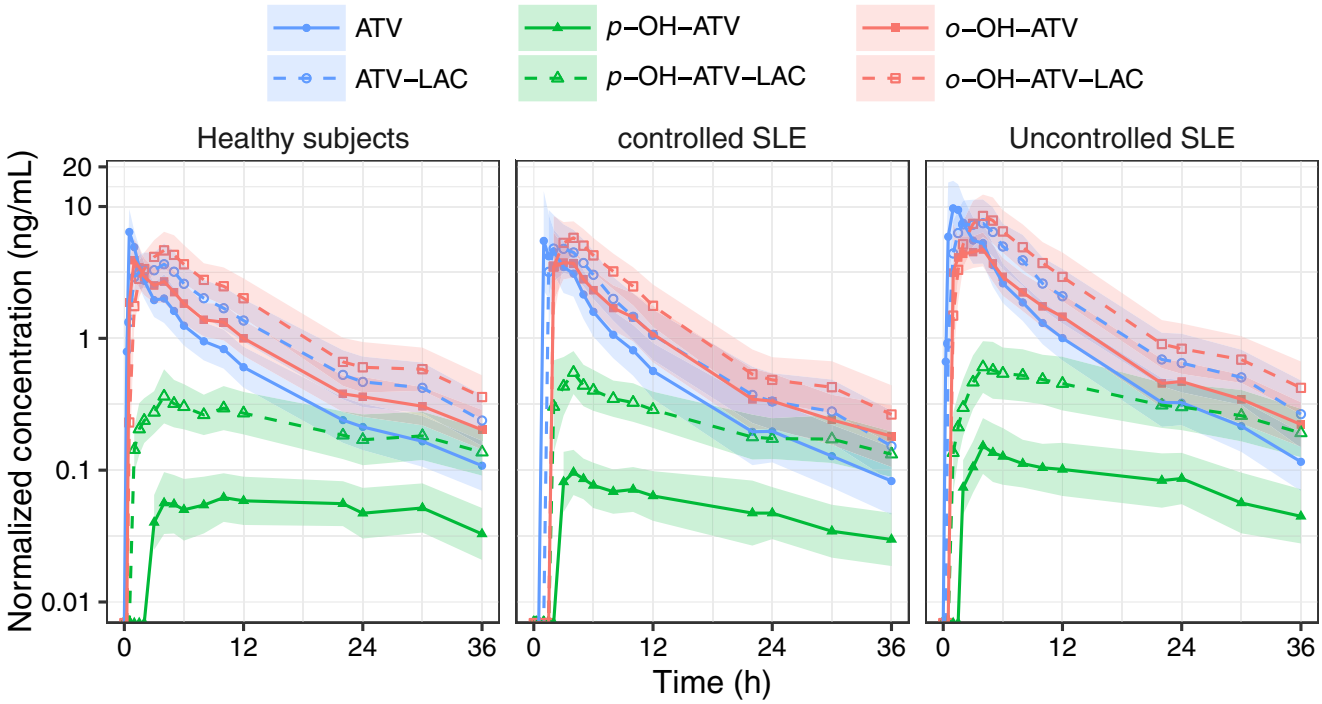

Figure 1 Plasma concentration curves of atorvastatin and its metabolites vs. time (0–36 hours) after a single oral dose of ATV in healthy subjects (n = 15), patients with controlled systemic lupus erythematosus (SLE, n = 13) and uncontrolled SLE (n = 12). Plasma concentrations were normalized to the 20 mg ATV dose. Data are presented as geometric means and 95% confidence intervals. ATV, atorvastatin; ATV-LAC, atorvastatin lactone; *p*-OH-ATV and *o*-OH-ATV, para-hydroxy and ortho-hydroxy atorvastatin; *p*-hydroxy and *o*-OH-ATV-LAC, para-hydroxy and ortho-hydroxy atorvastatin lactone.

Table 3 Pharmacokinetic parameters of atorvastatin for healthy subjects (n = 15), patients with controlled SLE (n = 13), and patients with uncontrolled SLE (n = 12)

Parameters	Healthy subjects	Controlled SLE	Uncontrolled SLE
$AUC_{0-\infty}$ (ng·hour/mL)	30.56 (22.69–41.15)	35.87 (24.53–52.45)	60.47$^{\triangle}$ (43.76–83.56)
C_{max} (ng/mL)	8.56 (6.42–11.40)	10.82 (8.17–14.31)	15.83$^{\triangle}$ (9.65–25.95)
T_{max} (hour)	0.73 (0.49–1.09)	0.79 (0.57–1.09)	0.92 (0.66–1.28)
$t_{½}$ (hour)	12.14 (8.23–17.89)	9.26 (7.70–11.15)	10.58 (7.19–15.59)
CL/F (L/hour)	654.5 (486.00–881.4)	557.6 (381.3–815.3)	330.7$^{\triangle}$ (239.30–457.00)
V_d/F (L)	7,159 (4,904–10,450)	3,917 (2,673–5,739)	2,609$^{\triangle}$ (1,607–4,234)
f_u (%)	8.68 (6.96–10.82)	8.60 (7.54–9.82)	8.02 (6.21–10.35)

Pharmacokinetic parameters are normalized to atorvastatin 20 mg dose and are presented as geometric means and 95% confidence intervals.
$AUC_{0-\infty}$, area under the plasma concentration vs. time curve extrapolated to infinity; CL/F, apparent total clearance; C_{max}, maximum observed plasma concentration; f_u, unbound fraction; SLE, systemic lupus erythematosus; T_{max}, time to reach C_{max}; $t_{½}$, elimination half-life; V_d/F, apparent volume of distribution. $P < 0.05$ analysis of variance: $^{\triangle}$(healthy subjects vs. uncontrolled SLE), no differences were observed between healthy subjects vs. controlled SLE or controlled SLE vs. uncontrolled SLE.

alpha-1-acid glycoprotein, AST, fasting blood glucose, total cholesterol, triglycerides, and LDL, as well as a slight reduction in hemoglobin and hematocrit values. Alterations in biochemical and hematological tests observed in the investigated patients with uncontrolled SLE are usual in this stage of the disease and may or may not be immune-mediated.[5]

Plasma concentrations of cytokines MCP-1 and TNF-α assessed on the first day of the study (D1; **Table 2**) are increased in the controlled SLE and uncontrolled SLE groups when compared with the healthy subjects group. Plasma IL-10 concentrations are also increased in the uncontrolled SLE group as compared to the healthy subjects group (**Table 2**). Asanuma *et al.*[4] also reported that patients with SLE exhibit increased plasma concentrations of MCP-1, a cytokine that correlates with coronary risk factors. According to McCarthy *et al.*,[5] increased plasma TNF-α concentrations are related to disease activity. In contrast, increased plasma IL-10 concentrations are related to a protective effect of the disease progression (i.e., IL-10 promotes B lymphocyte proliferation in the early course of the disease).

However, there were no significant differences in the plasma concentrations of the cytokines MCP-1 and TNF-α between controlled and uncontrolled SLE groups. At the time of blood sampling, all included patients with SLE were

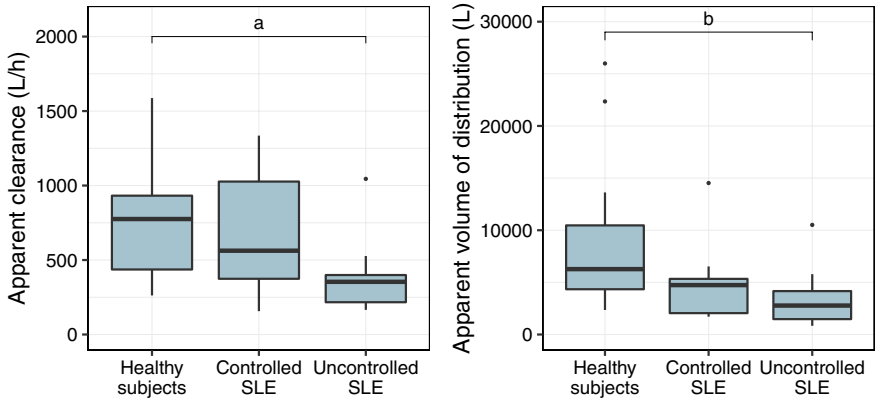

Figure 2 Boxplots of the apparent total clearance (L/hour) and apparent volume of distribution (L) of atorvastatin in healthy subjects (n = 15), patients with controlled systemic lupus erythematosus (SLE; n = 13) and uncontrolled SLE (n = 12). Data are presented as median, interquartile interval, and maximum and minimum values. $P < 0.05$ analysis of variance with Tukey's *post hoc* test.

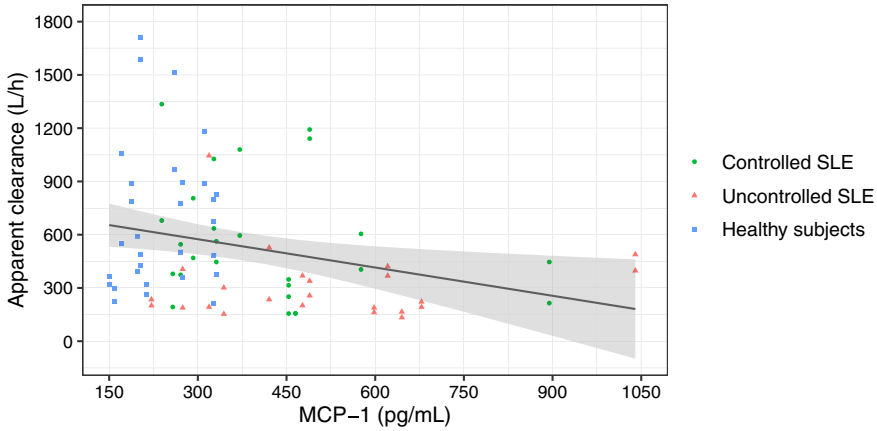

Figure 3 Linear regression analyses between apparent total clearance (L/hour) of atorvastatin and cytokine MCP-1 plasma concentrations (P = 0.01088) in all participants (n = 40); line: linear regression, shade: 95% confidence interval. SLE, systemic lupus erythematosus.

on stable doses of oral prednisone (5–40 mg/day). All included patients with controlled SLE were treated with 5 mg of prednisone, but some patients with uncontrolled SLE required higher doses (5 mg (n = 5); 10 mg (n = 3); 20 mg (n = 2); and 40 mg (n = 2)). The use of different prednisone doses between the controlled and uncontrolled SLE groups, associated to the fact that glucocorticoids decrease the synthesis of pro-inflammatory cytokines, such as IL-2, IL-6, and TNF-α,[39] could explain the observation of the absence of significant differences in the plasma concentrations of the cytokines between controlled and uncontrolled SLE groups.

In vivo CYP3A4 activity was evaluated for all participants using the oral clearance of MDZ to assess both gut and hepatic CYP3A4 activity. Although total ATV clearance is determined by the sinusoidal efflux clearance (CL^{s}_{ef}), and not CYP3A4-dependent CL_m, inhibition of CYP3A4 could potentially result in increased hepatic exposure to ATV.[24]

The oral clearance of MDZ values varied by 4–5-fold among healthy subjects, controlled, and patients with uncontrolled SLE (**Table 1**). Based on the oral clearance of MDZ values of 10–40 mL/minute/kg reported by Lamba *et al.*,[40] none of the participants had the apparent total clearance of MDZ values lower than 10 mL/minute/kg. The apparent total clearance of MDZ data indicates that there is no inhibition of gut and hepatic CYP3A4 in either the controlled SLE or the uncontrolled SLE groups. It should be noted that CYP3A4 inhibition could have occurred mainly in patients with uncontrolled SLE due to inflammatory activity and unregulated cytokine production, which may result in the downregulation of metabolizing enzymes. However, the comedication could have contributed to the observed higher values of MDZ clearance, especially in the uncontrolled SLE group. It is also observed that the apparent total clearance of MDZ values did not differ between the investigated groups ($P > 0.05$, ANOVA with Tukey's *post hoc* test), which suggests that CYP3A4-dependent ATV metabolism did not vary among the three groups.

The single oral administration of ATV (20, 40, or 80 mg) to the healthy subjects group (n = 15; **Table 3** and **Figure 1**) resulted in pharmacokinetic parameters of unchanged ATV similar to those reported by Huang *et al.*[41] in the investigation of healthy subjects treated with a single oral dose of 10 mg ATV.

The pharmacokinetic parameters of ATV were altered in patients with uncontrolled SLE when compared with the healthy subjects group (**Table 3**). The uncontrolled SLE group showed higher area under the curve from zero to

Table 4 Pharmacokinetic parameters of atorvastatin metabolites for healthy subjects, patients with controlled SLE, and patients with uncontrolled SLE

	p-OH-ATV	*o*-OH-ATV	ATV-LAC	*p*-OH-ATV-LAC	*o*-OH-ATV-LAC
Healthy subjects (*n* = 15)					
$AUC_{0-\infty}$ (ng·hour/mL)	—	40.42 (30.37–53.79)	49.21 (34.89–69.42)	—	64.09 (46.27–88.79)
$AUC_{0-36\ hours}$ (ng·hour/mL)	1.68 (1.04–2.72)	36.66 (27.57–48.75)	45.62 (32.58–63.89)	8.13 (5.55–11.90)	58.93 (42.80–81.13)
$AUC_{metabolite/atorvastatin}$	0.06 (0.04–0.07)	1.32 (1.10–1.59)	1.49 (1.22–1.83)	0.28 (0.21–0.36)	2.10 (1.63–2.69)
C_{max} (ng/mL)	0.10 (0.06–0.15)	5.53 (4.05–7.55)	4.33 (3.14–5.98)	0.42 (0.27–0.66)	5.05 (3.54–7.19)
T_{max} (hour)	4.57 (1.99–10.50)	1.14 (0.73–1.79)	2.40 (1.63–3.51)	2.94 (1.91–4.51)	3.73 (3.08–4.50)
$t_{½}$ (hour)	18.02 (12.29–26.42)	10.67 (8.77–12.99)	9.34 (8.31–10.50)	27.41 (16.00–46.96)	8.79 (7.73–10.00)
Controlled SLE (*n* = 13)					
$AUC_{0-\infty}$ (ng·hour/mL)	—	47.90 (35.23–65.12)	49.43 (32.77–74.57)	—	66.67 (49.37–90.03)
$AUC_{0-36\ hours}$ (ng·hour/mL)	2.08 (1.40–3.09)	44.48 (32.90–60.14)	47.18 (31.49–70.67)	9.23 (6.60–12.90)	62.54 (46.90–83.39)
$AUC_{metabolite/atorvastatin}$	0.06 (0.05–0.07)	1.34 (1.08–1.66)	1.32 (1.04–1.66)	0.25 (0.20–0.31)	1.86 (1.45–2.38)
C_{max} (ng/mL)	0.13 (0.08–0.20)	6.95 (5.49–8.81)	6.29 (3.97–9.97)	0.64 (0.44–0.94)	6.69 (4.92–9.09)
T_{max} (hour)	5.64 (2.98–10.67)	1.33 (0.80–2.22)	2.04 (1.55–2.67)	3.76 (2.79–5.06)	3.48 (2.79–4.35)
$t_{½}$ (hour)	16.48 (11.28–24.07)	9.39 (6.71–13.15)	7.97 (6.68–9.51)	20.15 (13.61–29.82)	8.56 (7.32–10.00)
Uncontrolled SLE (*n* = 12)					
$AUC_{0-\infty}$ (ng·hour/mL)	—	55.68 (42.18–73.52)	$98.74^{\triangle *}$ (74.31–131.20)	—	100.60 (72.40–139.70)
$AUC_{0-36\ hours}$ (ng·hour/mL)	3.25 (2.13–4.98)	52.02 (39.06–69.27)	$92.01^{\triangle *}$ (69.49–121.80)	13.34 (8.91–19.96)	93.35 (66.67–130.70)
$AUC_{metabolite/atorvastatin}$	0.05 (0.04–0.07)	$0.92^{\triangle *}$ (0.80–1.06)	1.33 (1.04–1.70)	0.23 (0.16–0.31)	1.66 (1.36–2.04)
C_{max} (ng/mL)	0.21 (0.11–0.40)	6.51 (4.50–9.42)	$9.08^{\triangle}$ (6.14–13.44)	0.70 (0.46–1.07)	8.86 (6.04–12.99)
T_{max} (hour)	3.72 (1.89–7.32)	1.84 (1.12–3.04)	2.47 (1.64–3.71)	5.48 (3.35–8.97)	3.90 (3.32–4.58)
$t_{½}$ (hours)	16.39 (10.69–25.15)	9.09 (7.42–11.13)	7.74 (5.87–10.21)	21.29 (13.53–33.50)	8.42 (6.95–10.21)

Pharmacokinetic parameters normalized to atorvastatin 20 mg dose are presented as geometric means and 95% confidence intervals.
— not determined; ATV-LAC, atorvastatin lactone; $AUC_{0-\infty}$, area under the plasma concentration vs. time curve extrapolated to infinity; $AUC_{0-36\ hours}$, area under the plasma concentration vs. time curve from 0 to 36 hours; $AUC_{metabolite/atorvastatin}$, metabolic ratios; C_{max}, maximum observed plasma concentration; *o*-OH-ATV, ortho-hydroxy atorvastatin; *p*-OH-ATV, para-hydroxy atorvastatin; *o*-OH-ATV-LAC, ortho-hydroxy atorvastatin lactone; *p*-OH-ATV-LAC, para-hydroxy atorvastatin lactone; T_{max}, time to reach C_{max}; $t_{½}$, elimination half-life.
$P < 0.05$ analysis of variance $^{\triangle}$(healthy subjects vs. uncontrolled SLE), *(controlled SLE vs. uncontrolled SLE), no differences were observed between healthy subjects vs. controlled SLE.

infinity ($AUC_{0-\infty}$; 60.47 vs. 30.56 ng·hour/mL), C_{max} (15.83 vs. 8.56 ng/mL) values, lower apparent total clearance (330.7 vs. 654.5 L/hour), and apparent volume of distribution (2,609 vs. 7,159 L) values when compared with the healthy subjects group; **Figure 2**.

Considering that the patients with uncontrolled SLE presented clinical and laboratory evidence of inflammation and showed increased plasma concentrations of cytokines (IL-10, MCP-1, and TNF-α; **Table 2**) involved in downregulation of membrane transporters,[1,25] the data allow us to infer that uncontrolled SLE reduces the activity of the sinusoidal uptake transporter OATP1B1, with consequent reduction of apparent volume of distribution and increased systemic exposure to ATV. The data presented in **Table 3** also show that changes in ATV pharmacokinetics depend on disease progression, as no changes were observed in the patients with controlled SLE group when compared with the healthy subjects group. It is important to highlight that both controlled and uncontrolled SLE groups have the disease, but only the patients from the uncontrolled SLE group had clinical and laboratory evidence of inflammation at the time of the investigation.

The unbound fraction of ATV (**Table 3**) found for the healthy subjects group (8.68%), controlled SLE group (8.60%), and uncontrolled SLE group (8.02%) reflect the literature data of ATV binding to plasma proteins (> 90%).[19] The data confirm that the values of the unbound fraction of ATV did not differ ($P > 0.05$, ANOVA with Tukey's *post hoc* test) among the investigated groups, demonstrating that the controlled SLE or uncontrolled SLE status does not change the unbound fraction of ATV.

The comparison of the systemic exposure to ATV metabolites between the healthy subjects and the uncontrolled SLE groups (**Table 4**) shows that uncontrolled SLE only presents increased $AUC_{0-36\ hours}$ (92.01 vs. 45.62 ng·hour/mL) and C_{max} (9.08 vs. 4.33 ng/mL) values for the inactive metabolite ATV-LAC. Considering the data presented in **Table 1**, regarding the apparent total clearance of the probe drug MDZ by CYP3A4, it is possible to enunciate that the CYP3A4-dependent presystemic elimination capacity of ATV-LAC does not differ between the uncontrolled SLE and the healthy subjects groups. Therefore, the higher plasma concentrations of ATV-LAC metabolite observed in the uncontrolled SLE group cannot be explained by metabolism, but can be explained by the reduction in the activity of the sinusoidal uptake transporter OATP1B1, given that ATV-LAC is more basic and is more lipophilic than the acid form, but also dependent on OATP1B1 to some extent. *In vitro* studies of inhibition of OATP1B-mediated uptake of estradiol 17β-D-glucuronide, the ATV-lactone was a less potent inhibitor compared with ATV acid form (half-maximal inhibitory concentration values threefold higher for the ATV-lactone compared with ATV acid form).[18]

The pharmacokinetics of ATV and its metabolites did not differ between patients with controlled SLE and healthy

subjects groups (**Tables 3 and 4**), inferring that the inflammation, rather than the disease itself, is responsible for the changes described in the uncontrolled SLE group as a consequence of the inhibition of the activity of the sinusoidal uptake transporter OATP1B1.

The AUC metabolic ratios of active metabolite/unchanged drug observed in the present study for the healthy subjects group (**Table 4**) were 0.06 for *p*-OH-ATV and 1.32 for *o*-OH-ATV, whereas for the inactive metabolites were 1.49 for ATV-LAC, 0.28 for *p*-OH-ATV-LAC, and 2.10 for *o*-OH-ATV-LAC. Few studies report the pharmacokinetic parameters of active and inactive ATV metabolites alike. The study by Hermann *et al.*[42] conducted in healthy subjects treated with multiple doses of 10 mg of ATV for 1 week shows AUC metabolic ratios close to the ones reported in the present study for the active (0.11 for *p*-OH-ATV, and 0.54 for *o*-OH-ATV) and inactive metabolites (2.54 for ATV-LAC, 0.42 for *p*-OH-ATV-LAC, and 1.85 for *o*-OH-ATV-LAC). Other single oral dose administration studies of ATV reported only the metabolic ratios of AUC for the active metabolite *o*-OH-ATV, with values of 1.25,[41] 1.19,[43] or 0.77[44] and, therefore, close to the value of 1.32 reported in the present study.

The correlation between the apparent total clearance of ATV and the plasma cytokines evaluated in the present study (INF-γ, IL-10, IL-1β, IL-6, IL-8, MCP-1, and TNF-α; **Table 2**) was only significant (P = 0.01088) for MCP-1, as it can be seen in **Figure 3**. The apparent total clearance of ATV was higher in patients with low MCP-1 plasma concentrations, such as those seen in the healthy subjects group, whereas the lowest apparent total clearance of ATV values were observed in patients with higher plasma concentrations of MCP-1, such as those observed in the uncontrolled SLE group.

Study limitations include the use of different ATV doses, the absence of enrolled patients diagnosed with SLE with SLEDAI scores higher than 15, the absence of pharmacodynamic parameters to assess ATV liver exposure, and the absence of OATP1B1 genotype data.

In conclusion, uncontrolled SLE (SLEDAI 6–15) increases the systemic exposure to ATV and ATV-LAC, inferring inhibition of the sinusoidal uptake transporter OATP1B1 activity, once *in vivo* CYP3A4 activity assessed by oral clearance of MDZ was unchanged. The inflammation, not the disease, is responsible for the changes described in the uncontrolled SLE group as a consequence of the inhibition of the activity of the sinusoidal uptake transporter OATP1B1 because systemic exposure to ATV and its metabolites was not altered in the patients with controlled SLE group. Thus, the progression of the disease is clinically relevant for dose adjustments of OATP1B1 substrates.

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