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Pharmacokinetic and bioequivalence testing of atorvastatin formulations in healthy male volunteers

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The aim of this study was to compare the bioavailability of two atorvastatin formulations (Divator® Drogsan Pharmaceuticals, Ankara, Turkey, as the test formulation, and Lipitor®, Pfizer Ireland Pharmaceuticals, Dublin, Ireland, as the reference formulation) in 52 healthy volunteers. The study was conducted using a randomised, single-dose, two-way crossover study with a 2-week washout period between the doses. Since the 90% confidence intervals for $C_{max,}$ AUC_{0-72} and $AUC_{0-\infty}$ ratios for both, the parent atorvastatin and its main active metabolite ortho-hydroxy atorvastatin, were within the pre-defined Bioequivalance acceptance limits approved by EMEA, we concluded that the atorvastatin formulation elaborated by Drogsan Pharmaceuticals, was bioequivalent to the Lipitor in its rate and extent of absorption.

The HMG-CoA-reductase inhibitor atorvastatin as well as some of its active metabolites are pharmacologically active in humans. Approximately 70% of circulating inhibitory activity for 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) is attributed to active metabolites. A high-performance liquid chromatography tandem mass spectrometry (LC/MS/MS) method has been reported for the quantitative determination of atorvastatin and its metabolites (Bullen 1999). Elimination of this drug is mainly through the bile; renal excretion of radiolabelled drug and metabolites in urine is negligible (Lea and McTavish 1997).

The first objective of this study was to evaluate, in healthy volunteers, the bioequivalence (BE) of a test formulation of the 40 mg film tablet of the HMG-CoA reductase inhibitor atorvastatin (Divator, Drogsan, Turkey) and a commercial formulation of 40 mg (tablets) of atorvastatin (libitor®, Pfizer, Ireland) as reference formulation.

Because the major active metabolites of atorvastatin have an activity similar to that of the parent compound, a second objective of this study was to analyse the pharmacokinetic profile of *ortho*-hydroxy atorvastatin (o-AT) and *para*-hydroxy atorvastatin (p-AT).

Because little is known about the pharmacokinetic profile of the atorvastatin main metabolites and due to the high variability of the atorvastatin (Lea and McTavish 1997), a large number of subjects (52) were enrolled in a single cross-over two way study design. For the same considerations, the use a wide 90% confidence interval (75% to 133%) to C_{max} was justified, which is within the acceptance limits by EMEA.

All subjects completed the study except one, who was excluded after the first pharmacokinetic phase, due to acute increase of hepatic enzymes related to a hepatic infection. None of the data for this subject were included in the analysis.

The plasma drug concentration-time curves show that the mean concentrations of unchanged atorvastatin and the active metabolite, *o*-AT, were similar for the two formulations over the 72-hr sampling period (Fig.). The majority of the plasma levels of p-AT from test and reference medications were under the level of detection of the method used. Therefore, the data from *p*-AT was not analysed and not used for supporting bioequivalency.

The results after statistical analysis of the main pharmacokinetic parameters from both, parental and o-AT, are shown in the Table. The parametric 90% confidence intervals for the main pharmacokinetic parameter values of $C_{max,}$ AUC_{0-72} and $AUC_{0-\infty}$ lie entirely within the BE acceptance limits approved by EMEA (i.e. 80%–125% to AUC_{0-72}, $AUC_{0-\infty}$ and 75%–133% to C_{max}). The C_{max} intra-subject coefficients of variability were 35.45% and 34.09% to atorvastatin and *o*-AT, respectively. The point estimate (test/reference geometric mean $\times 100$) of C_{max}, atorvastatin and *o*-AT, fell within the 80% to 125% BE limits (see Table). These results confirmed that atorvastatin belongs to the group of highly variable drugs, which support a large number of volunteers, scaling from a 2-period to a replicate period and wider acceptance limits of C_{max} in its BE study design.

Maximal atorvastatin levels of the test and reference were observed after 0.88 ± 0.42 and 0.69 ± 0.38 h, respectively. The mean half-lives ($t_{1/2}$) were 9.18 ± 2.45 h for the test and 9.07 ± 2.71 h for the reference.

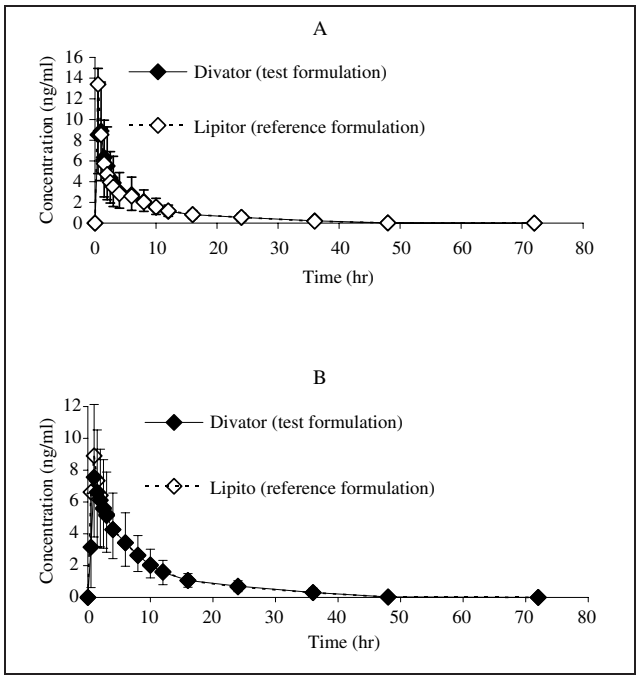

Fig.: Mean concentrations of unchanged atorvastatin (A) and the active metabolite ortho-hydroxy atorvastatin (B) in the plasma of 51 subjects after the oral administration of sample doses (40 mg) of two different atorvastatin formulations, namely, Divator and Lipitor

Table: Arithmetic and geometric means and 90% confidence intervals (90% C.I.) of C_{max}, AUC_{0-72} and $AUC_{0-\infty}$ (log transformed) of atorvastatin and its main active metabolite (o-AT) during single dose administrations of 40-mg test and reference formulations in healthy male subjects

	Arithmetic mean		Geometric mean		90% C.I.	Acceptable range	Point estimate (%)
	Test	Reference	Test	Reference			
Atorvastatin							
C_{max}	11.76	14.54	10.94	12.95	75.12– 95.04	75–133	84.48
AUC_{0-72} (ng · h/ml)	53.76	54.07	48.99	49.38	94.64–104.01	80–125	99.21
$AUC_{0-\infty}$* (ng · h/ml)	57.17	57.74	52.42	53.00	94.59–103.38	80–125	98.91
o-AT							
C_{max} (ng/ml)	9.03	10.45	8.05	9.01	79.31–100.73	75–133	89.35
AUC_{0-72} (ng · h/ml)	63.36	65.73	58.24	60.84	91.37–100.29	80–125	95.73
$AUC_{0-\infty}$* (ng · h/ml)	68.74	69.57	64.14	64.99	93.98–102.64	80–125	98.69

* 89.4% of the $AUC_{0-\infty}$ was measured

Maximal o-AT levels of the test and reference were observed after 0.79 ± 0.41 and 1.27 ± 0.99 h, respectively. The mean half-lives ($t_{1/2}$) were 9.51 ± 2.04 h for the test and 9.11 ± 1.93 h for the reference.

In summary, regarding C_{max} and $AUC_{0-\infty}$ of atorvastatin and its main active metabolite *o*-AT, the BE of both formulations of 40-mg atorvastatin tablets was concluded. Therefore, these two formulations can be used interchangeably in clinical practice.

Experimental

1. Study design and clinical protocol

Fifty-two healthy adult male volunteers aged between 18 and 50 years (27.57 ± 7.45 years, mean ± S.D.; range 20–49) and within the 15% of the ideal body weight, weight between 58.0 and 95.0 kg (78.20 ± 7.30) and height between 168 and 192 cm (180.69 ± 5.16), were selected for the study. All subjects gave written informed consent and the University Hospital of Olomouc ethics committee approved the clinical protocol. All volunteers were healthy assessed by medical history, clinical examination, blood pressure, ECG and laboratory investigation (haematology, blood biochemistry and urinalysis). No subject had a history or evidence of hepatic, renal, gastrointestinal, or haematological deviations, or any acute or chronic disease or drug allergy.

The study was conducted in a randomised, single-dose, two-way crossover study with a 2-week washout period between the doses. In the evening before the pharmacokinetic phases, the volunteers were admitted to the BE Test Unit. After overnight fasting, they received a tablet containing 40 mg of atorvastatin along with carbonated water (240 mL). All subjects were fasted for 2 h following drug administration, after which a snack was consumed. Standard meals were provided to volunteers 6 h after dosing. No other food was permitted during the hospitalisation period. Liquid consumption was permitted *ad libitum* 2 h after the drug administration but xanthine-containing drinks were prohibited.

The study was performed in accordance with the guidelines of the revised Declaration of Helsinki on biomedical research involving subjects and the requirements of Good Clinical Practice.

2. Formulations, sample collection and chromatographic conditions

The following formulations were employed: Divator 40 mg film tablet as test formulation, and Lipitor® 40 mg tablets as reference formulation.

Blood samples (9 ml) from a suitable antecubital vein were collected into a sodium-citrate containing tube before dosing and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, 24, 36, 48 and 72 h after administration the formulations (40 mg). The blood samples were centrifuged at 1800 rpm for 10 min at 4 °C, the plasma decanted and stored at −70 °C pending drug analysis. After a period of 14 days, the sampling was repeated in the same manner to complete the crossover design.

For analysis of plasma samples of atorvastatin, *o*-AT as well as *p*-AT, a validated LC/MS/MS method with electrospray ionisation in the positive mode (ESI(+)) and multiple reaction monitoring (MRM) was established. This advanced technique was regarded appropriately sensitive and reliable in order to determine atorvastatin and metabolites levels in plasma as low as 0.32 and 0.31 ng/ml, respectively.

HPLC separation (Jasco Germany, Groß-Umstadt, Germany) was carried out on an analytical column (Synergi Polar RP, 4 µm, 75*4.6 mm i.d.) using isocratic elution with acetic acid solution in acetronitrile, methanol and water mixture. The analysis was performed at a flow rate of 0.8 ml/min (split: 100 µl/min into MS). Triple stage quadrupole mass spectrometer Quatro Micro with API-source and ESI-probe (Micromass, Altrincham, GB) was employed. The standard of atorvastatin was purchased from Mikromol GmbH, Germany. The standards of o-AT and p-AT were purchased from SynFine Research, Ontario Canada. The calibration curves were linear over the range of 0.32–39.96 ng/ml for atorvastatin, 0.31–38.70 ng/ml for o-AT and 0.32–40.02 ng/ml for p-AT using 1.0 ml plasma samples. The precision of the method, expressed as coefficients of variation (CV%) for atorvastatin, *o*-AT and *p*-AT was 4.1, 1.7 and 4.7%, respectively, for samples containing 0.33, 0.34 and 0.33 ng/ml, respectively.

3. Pharmacokinetic and statistical analysis

Pharmacokinetic analysis was performed by means of a model-independent method using the GraphPad Prism (rev. 2.01) and Lotus Approach and Lotus 1-2-3 (rev. 9) computer programs. The maximum atorvastatin and o-AT plasma concentrations (C_{max}) and the corresponding time of peak plasma concentrations (T_{max}) were obtained directly from the plasma atorvastatin concentrations. The areas under the plasma concentration-time curve, AUC_{0-72} and $AUC_{0-\infty}$ were calculated by using the linear trapezoidal method. For the purpose of BE analysis, two-way analysis of variance (ANOVA, GLM procedure) was used to assess the effect of formulations, periods, sequences, and subjects on the raw (untransformed) and logarithmically transformed data of AUC_{0-72}, $AUC_{0-\infty}$, C_{max} and $t_{1/2}$. Then, the 90% confidence intervals of the test-reference ratios for AUC_{0-72}, $AUC_{0-\infty}$ and C_{max} (log transformed) were determined.

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