Abstract

The aim of this work was to study the effect of the sex and the smoking status on the pharmacokinetics and the bioequivalence assessment of a branded and a generic cyclosporine A microemulsion formulation in soft-gelatin capsule.

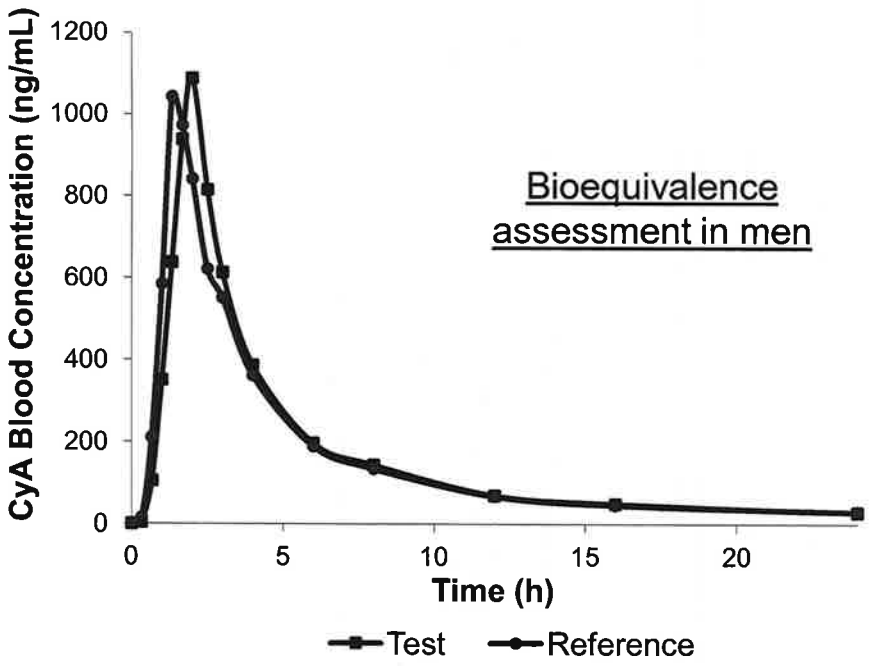
Sixteen healthy volunteers (eight women and eight men) participated in a CyA bioequivalence study, with nine of the volunteers being smokers. Sandimmun Neoral® (brand formulation; Reference) and Sigmasporin Microral® (generic formulation; Test) were administered under fasting conditions. Pharmacokinetic parameters were calculated through non compartmental analysis. Bioequivalence was declared based on the 90% confidence intervals (90% CI) for the T/R ratio of the geometric means for each parameter. In vitro determination of the capsules opening time was performed in simulated gastric fluid without enzyme with USP Apparatus 2.

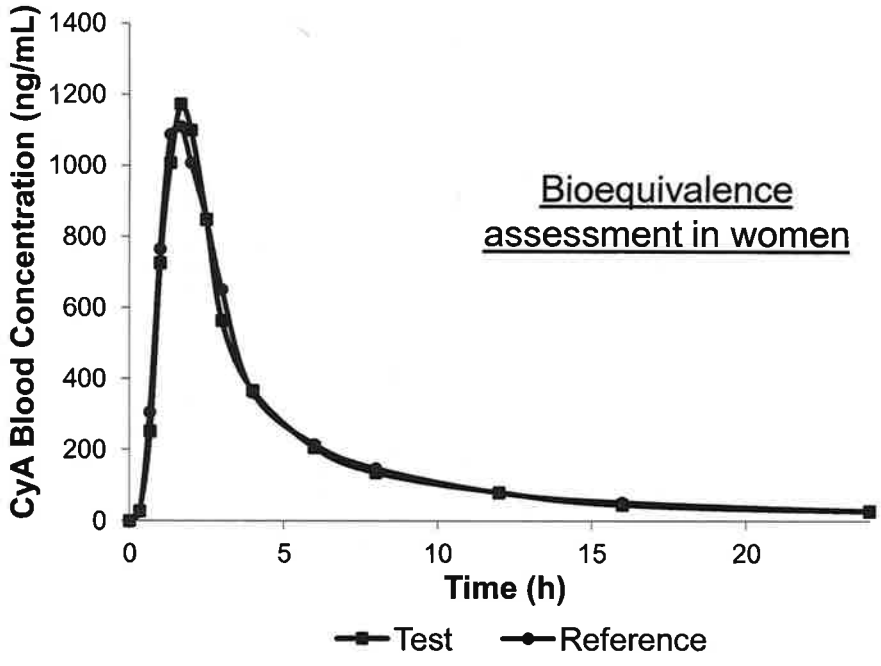
The extent of absorption was similar between both products for all subjects or each sex-group. The absorption rate was similar for both products when considering all subjects, whereas a significant difference in the Tmax between the two products was observed for the male subjects only, which relates to its slower capsule opening time observed in vitro (12.4 vs. 6.0 min). No differences were observed in women that could relate to their slower gastric emptying. Differences in drug exposure were observed between smokers and non-smokers.

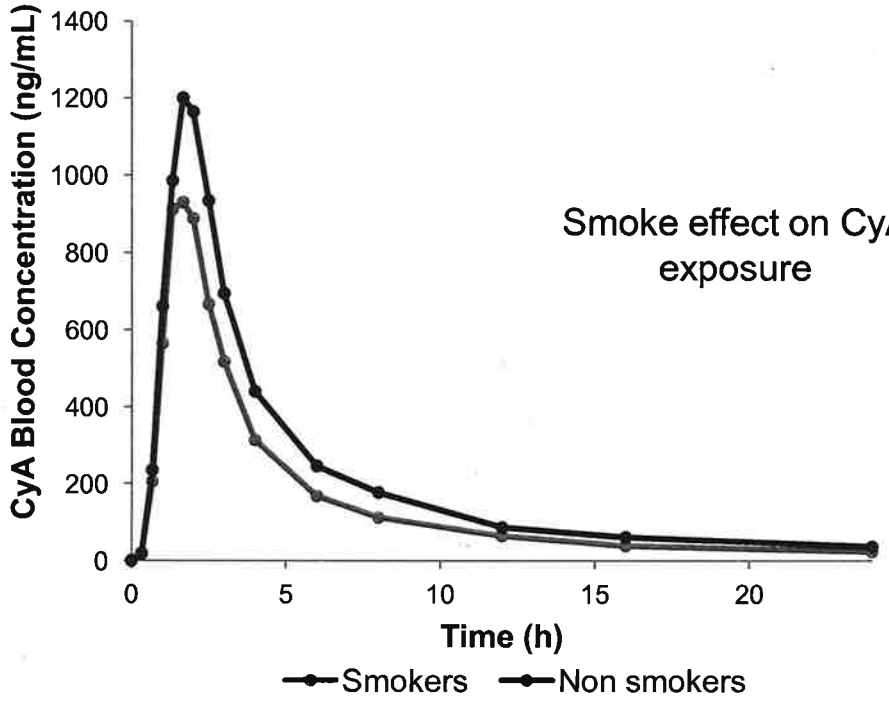
Sex- and smoke-related differences in the gastrointestinal transit should be considered when the on-set time would be determinant for the treatment success of a drug.

Keywords: sex; smoking; cyclosporine; gastrointestinal transit; microemulsion; bioequivalence

*Graphical Abstract (for review)







Sex- and smoke-related differences in gastrointestinal transit of cyclosporin A microemulsion capsules

Pietro Fagiolino[1*], Marta Vázquez[1], Manuel Ibarra[1], Laura Magallanes[1], Natalia Guevara[1], Nikoletta Fotaki[2]

(1) Department of Pharmaceutical Sciences - Faculty of Chemistry. Bioavailability and Bioequivalence Center for Medicine Evaluation. *Universidad de la República.* Uruguay.

(2) Department of Pharmacy and Pharmacology. Faculty of Science. University of Bath. United Kingdom.

(*) Corresponding author

Pietro Fagiolino.

Faculty of Chemistry. POBox 1157.

11800 Montevideo. Uruguay

Introduction

Cyclosporin A (CyA) is a non-ribosomal cyclic-neutral-hydrophobic peptide of eleven amino-acids, which is produced by the fungi *Tolypocladium inflatum Gams* and is extracted from the mycelia (Borel et al., 1995). The clinical usefulness of CyA is to prevent graft rejection following organ transplantation and also for the treatment of autoimmune diseases (Krensky et al., 2010; Sandimmune®, 2010).

CyA gastrointestinal absorption is incomplete and variable. Compared with an intravenous infusion, the absolute bioavailability of soft capsules is approximately 30% (Sandimmune®, 2010). This low and variable bioavailability is determined by many factors including: solubility, emulsification, and pre-systemic hepatic and intestinal metabolism, which is mediated by the concerted action of P-glycoprotein (P-gp) efflux transporter and the CYP3A4 isozyme, located in the liver and several extra-hepatic sites, most notably, the epithelium of the upper intestine (Christians et al., 2000; Fahr, 1993; Saeki et al., 1993; Wu et al., 1995; Kolars et al., 1991; Webber et al., 1992). Grevel et al. (1986) suggested the existence of an absorption window for CyA located in the upper portion of the intestine.

Microemulsion formulations (Sandimmun Neoral ®-Novartis, and several generic brands) have improved its therapeutic performance in comparison with the emulsion formulation (Sandimmun® - Novartis), and these formulations exhibited dose-proportionally in AUC over a wide and clinically relevant dosage range and an increase in the rate and extent of CyA absorption. The relative bioavailability of CyA after administration as a microemulsion ranges from 174 to 293 % compared to Sandimmun® (Mueller et al., 1994). Another benefit of the microemulsion formulation is the decreased inter-individual variability observed since CyA becomes ready to be absorbed once it is released from the soft capsules (Mueller et al., 1994; Qazi et al., 2006).

There is strong evidence supporting sex differences on gastric emptying, with female subjects having longer lag-periods for solid meals, lower gastric emptying rate and higher gastric residence time (Bennink et al., 1998; Datz et al., 1987; Hermansson et al. 1996; Hutson et al., 1989; Knight et al., 1997; Sadik et al., 2003). Differences were also reported for transit time throughout large intestine, which seems to be shorter in men (Sadik et al., 2003; Degen et al., 1996). Reports are controversial regarding transit time in small intestine, as some studies reported no sex differences while others found a significantly slower transit in females (Freire et al., 2011).

Sex-specific pharmacokinetic differences related with CyA metabolism have been also reported. On the one hand, a higher CYP3A4 activity observed in females (Wolbold et al., 2003; Greenblatt et al., 2008; Scandlyn et al., 2008) would decrease oral bioavailability and increase systemic clearance. On the other hand, only one-third to one-half of the P-gp expression observed in men was reported in females (Schuetz et al., 1995). Substrates of both CYP3A4 and P-gp have showed higher bioavailability and clearance in women (Schwartz et al., 2007). Consequently, these facts might preclude a similar CyA exposure between sexes.

Beyond the consequence that these differences may cause on drug clinical response, they can have a major impact on bioequivalence between oral formulations containing P-gp and CYP3A4 substrates due to the sex-by-formulation interaction. Men and women can have different capabilities to discriminate two drug-products (Chen et al., 2000; Ibarra et al., 2012), a phenomenon usually neglected in conventional average bioequivalence studies. Kees and coworkers (2007) conducted two crossover studies in which effects of two formulations and food intake on CyA bioavailability were studied, and found that food significantly increased maximum concentration only in male subjects.

Tobacco smoking has also been related with effects on gastric physiology, although debate persist as some studies reported a delayed gastric emptying of solids for smokers (Nowak et al., 1987; Miller et al., 1989; Johnson et al., 1991) while others associated tobacco and nicotine with increased gastric motility and faster emptying rates of solid and liquid contents (Ferreira et al., 2002; Grimes and Goddard, 1987; Sanaka et al., 2005; Hanson and Lilja, 1987; Graff et al., 2001). Graff et al (2001) and Lagrue et al. (2006) also found an increased intestinal peristalsis secondary to nicotine intake.

A pharmacokinetic analysis on data coming from a CyA bioequivalence study will be presented here below. The aim of this work was to study whether the sex and the smoking status of individuals could affect both the pharmacokinetics and the bioequivalence assessment between two CyA soft-capsulated microemulsion formulations.

Materials and Methods

Materials

1. Equipments

GFL Destilator 2008 (Germany), Distek dissolution apparatus 2100C (NJ, United States), Oakton ph6 pH-meter (IL, United States), and Abbott AxSYM™ system for immunoassays (Abbott laboratories, IL, United States) were used.

2. Chemicals

Following reagents were used: hydrochloric acid from Dorwil (BA, Argentine) and potassium chloride from Carlo Erba (MI, Italy). Distilled water was obtained in our laboratory. Abbott

reagents for AxSYM CyA determination based on fluorescence polarization immunoassay (FPIA) were provided by Bioerix (MVD, Uruguay).

Assayed formulations were soft gelatin capsules containing 100 mg CyA microemulsion: Test (T) Sigmasporin Microral® (lot number 408935), EMS Sigma Pharma, Brazil; and Reference (R) Sandiummun Neoral® (lot number S0181) Novartis, Switzerland.

Methods

1. *In vitro* determination of the capsules opening time

Six units of each product were tested. The *in vitro* assay was performed using the simulated gastric fluid without enzyme (aqueous solution of HCl and KCl, pH 1.2), according to the United States Pharmacopoeia (USP, 2009). This medium is considered biorelevant by the World Health Organization (WHO, 2006). The conditions were: USP Apparatus 2 (paddle); 50 rpm stirring speed; volume 500 mL; temperature 37±0.5 ºC. Each dosage unit was visually inspected up to the capsule shell rupture time. This time was recorded unit by unit in separated assays.

2. Bioequivalence Study

2.1 Subjects and study design

Sixteen Caucasian healthy volunteers (eight women and eight men) between 18 and 37 years old with mean body weight (SD) of 58 (7.5) and 82 (12) kg, respectively, were enrolled in a CyA bioequivalence study. Nine of the volunteers were smokers (4 men and 5 women) and seven non-smokers (4 men and 3 women). The study was carried out with two capsules (100 mg) of CyA: Test formulation (T) or Reference formulation (R), administered under fasting conditions with 100 mL of water, in a two-period and two-sequence (TR and RT), randomized, compensated-crossover design. A two-week washout period was kept between both

administrations. The study protocol and informed consent form were designed according to the ethical guidelines for human clinical research and were approved by the Institutional Ethics Review Committee of the Faculty of Chemistry (Uruguay). Written informed consent was obtained from all subjects before their entry in the study. The study was performed in the Bioavailability and Bioequivalence Centre for Medicine Evaluation, situated in "Dr. Juan J. Crottogini" Hospital (Montevideo, Uruguay).

Volunteers came to the Centre the first day of each week, with an eight-hour overnight fasting period. Standardized meals (lunch, tea, dinner and breakfast) were provided at three, seven, eleven and twenty-two hours after dose administration. Twenty-four hours post-dose, the volunteers were released from the Centre, returning at thirty-four and forty-five hours post-dose for blood sampling. During the second day of the study, there were no food restrictions.

2.2 Sampling and chemical analysis

Blood samples were drawn from the antecubital vein through cannulation and placed into heparinized tubes immediately. The samples were drawn before dosing (0 h) and at 0, 0.33, 0.67, 1, 1.33, 1.67, 2, 2.5, 3, 4, 6, 8, 12, 16, 24, 34, and 45 hours after dosing. Blood samples were stored refrigerated (2 – 8ºC) until analysis.

Quantification of CyA in blood was done by Fluorescence Polarization Immuno Assay (FPIA) using AXSYM (Abbot™) equipment, according to the instructions given in the package insert. The lower limit of quantification was determined to be 12.5 ng/mL, since intra-and-inter-day precision was below 20%, in terms of coefficient of variation, and accuracy was comprised between 85 and 115%.

2.3 Pharmacokinetic Analysis

Non-compartmental pharmacokinetic analysis was performed using the software tool Kinetica 5.0 (Thermo Fisher Scientific, Waltham, MA).The maximum CyA blood concentration (C_{MAX}) and the time-to-peak (T_{MAX}) were computed for each volunteer from experimental data. The area under the blood concentration–time curve from zero to infinite (AUC_{inf}) was calculated using the trapezoidal rule until the last quantifiable concentration (C_{LAST}): AUC_T, and extrapolated to infinitive adding the term C_{LAST}/k_{EL}, being k_{EL} the first order elimination rate constant calculated from the slope of the log-linear concentration-time regression of data collected 12 h post-dose. The half-lives ($t_{1/2}$) for both drugs were calculated as $Ln(2)/k_{EL}$. C_{MAX}/AUC_{inf} ratio was calculated for each volunteer as an estimator of the absorption rate (Bois et al., 1994; Schall et al., 1994; Tothfalusi and Endrenyi, 1995).

2.4 Statistical Analysis

Pharmacokinetic parameters (C_{MAX}, AUC_{inf} and AUC_T), in logarithmic scale, were processed by analysis of variance (ANOVA, Microsoft Office Excel 2007 software) considering subjects, sequences, periods and treatments as variation sources. Coefficient of variation (CV) of the ANOVA was calculated according to the Equation 1:

$$CV = 100\sqrt{e^{S^2} - 1} \qquad \text{(Equation 1)}$$

Being S^2 the residual variance of the ANOVA performed on the log-transformed parameters.

A T-Wilcoxon test was used to evaluate the Test-Reference difference for T_{MAX}, since a non-normal distribution was assumed. Bioequivalence between Test and Reference was declared if

the 90% confidence intervals (90% CI) for the T/R ratio of the geometric means for each parameter were contained within the range of 0.80–1.25, and T_{MAX} did not differ significantly. This procedure was carried out for the total of volunteers and for each sex group.

3. Smoker versus non-smoker comparison

3.1 Pharmacokinetic Analysis

The exposure parameters C_{MAX} and AUC_{inf} were corrected by the subject body weights in order to override differences related with the size of individuals. Neither k_{EL} nor C_{MAX}/AUC_{inf} were corrected since these parameters reflect either the elimination or the absorption rate constant, respectively.

3.2 Statistical Analysis

Non-paired Student t-tests were carried out (Microsoft Office Excel 2007 software) for comparing normal (non log-transformed) pharmacokinetic parameters between smokers and non-smokers.

Results

1. Capsules opening time

The dissolution assay carried out with six capsules of each product showed that the Test product presents a significantly slower capsule opening time (12.4±5.0 min) than the Reference (6.0±2.4 min).

2. Bioequivalence study

Table I shows the pharmacokinetic parameters of CyA after Test and Reference administration. The results are shown for each subject. Mean plasma concentration profiles of CyA (male and female) are shown in Figs. 1 and 2, respectively. Tables II to IV show the Test/Reference (T/R) geometric mean ratios for the pharmacokinetic parameters considering male, female, and all subjects, respectively.

Regarding the bioequivalence between formulations, the Test could be assessed as bioequivalent with the Reference. The extent of absorption was similar between both products as shown by the inclusion of the 90% CI of AUC_{inf} geometric mean ratio between 0.80 and 1.25, either considering the whole subjects or each sex-group (Tables II-IV). Similarly, the absorption rate of formulations was assessed as bioequivalent considering the geometric mean ratio of C_{MAX} and C_{MAX}/AUC_{inf}. No significant difference between formulations was obtained for T_{MAX} when all the individuals were considered. However, in male subjects (see Table I), T_{MAX} of the Reference was significantly lower than that of the Test ($p<0.02$). Women did not show any differences between formulations regarding T_{MAX}.

Considering the sex of individuals, AUCs and C_{MAX} differed, but once they were corrected by subject body weights such differences disappeared. Hence, no sex-related differences in the apparent clearance could be assessed when the same dose by kilogram is considered. As it can be seen in Table I, similar to men (average between formulations = 14.4 h) was the half-life found in women (average between formulations = 15.0 h).

3. Smoking effect

Since several individuals had the habit of consuming tobacco, a comparison between smoking (SMK) and non-smoking (NSMK) subjects was performed (Table V). Differences were observed in either AUC_{inf} or AUC_{inf} corrected (multiplied) by body weight revealing that although the

dose has been normalized, differences in drug exposure still persist. Conversely, C_{MAX} did not display any differences between both groups of individuals. Regarding the velocity of drug elimination, k_{EL}, no differences between SMK and NSMK were detected in both formulations.

Figure 3 shows blood CyA mean concentration profiles obtained in SMK and NSMK subjects. As it can be seen, lower AUC and faster T_{MAX} were observed in smokers. Corrected C_{MAX} was significantly reduced in smokers when formulation Test was given, but no difference was observed when Reference was administered. Conversely, the Reference, but not the Test, was able to detect differences between SMK and NSMK in C_{MAX}/AUC_{inf} (Table V).

Discussion

1. Bioequivalence and sex-related differences in the gastrointestinal tract

The results obtained in the male group (Table I) showed that T_{MAX} for the Test (2 h) was higher than for the Reference (1.33 h), which would be in accordance with its slower capsule opening time observed in vitro (12.4 *vs.* 6.0 min). However, in women no differences were observed. This fact could be related with a slower gastric emptying in females. Residence time in the stomach might be prolonged in females, giving the Test product enough time to open the capsule and thereafter to release the microemulsion. Hence, drug delivery to the gut would be done by the Test in a similar extent and rate than the Reference. Conversely, as the stomach-residence time is shorter in men, Test product would not have enough time to release the microemulsion at the same extension that the Reference when the gastric content flows to the duodenum. So, a delay in T_{MAX} for the Test might be registered in men (median of 0.67 h for the Test-Reference difference).

These facts would support the hypothesis that gastric emptying could just affect the time from where the CyA becomes available to pursuit the absorption through intestinal membranes. Then, in the case of men, as their higher strength of gastric contraction promotes shorter intervals between each gastric discharge, the faster the formulation releases the drug, the faster its intestinal absorption starts. Women, with longer intervals of gastric discharge, are unable to differentiate drug passage from the stomach to the intestine when formulations have small differences in drug release.

If C_{MAX} is considered, a greater variability (CV) was observed in women than in men (Tables II and III). Since this variability was also observed in C_{MAX}/AUC_{inf} but not in AUC, the rate of drug absorption should be the main source of variability in women.

Between-formulation T_{MAX} difference observed in men (40 min) was near 7-fold higher than the between-formulation capsule opening time difference (6.4 min) registered *in vitro*, when 500 mL of simulated gastric fluid was used. This could be related with the lower gastric fluid content and the slower stomach agitation when drug-products are administered in fasting condition (Hall, 2006).

A between-formulation difference of forty minutes in T_{MAX} found in men should not have clinical implications since AUCs and C_{MAX} did not differ after both products were administered. Nevertheless, it is important to highlight that these differences might be important in the clinical setting for other drugs in which the on-set time would be determinant for the treatment success.

Finally, it could be assessed for the population that Test and Reference are bioequivalent based on their similar C_{MAX} and AUC_{inf} values, and because of the 90%CI of the T/R geometric

mean ratios for both exposure parameters were included within the (0.80-1.25) bioequivalence interval (Table IV). To emphasize this conclusion it could be said that bioequivalence was also assessed in both male and female subjects (Tables II and III), and hence formulation interchangeability could be precluded regardless the sex of individuals.

2. Smoking effect on the gastrointestinal transit of formulations

Regarding the effect of smoking on the CyA pharmacokinetics, some interesting results were obtained. Significant differences were observed in AUC_{inf} between smokers and non-smokers, for both Test and Reference (Table V) that could relate to either a decrease in bioavailability or an increase in clearance. However, since no significant differences were found in the elimination rate constant (k_{EL}), the bioavailability, and not the clearance, might be affected by cigarette smoking. Ferreira et al. (2002) observed that nicotine increased gastric motility in rats by means of antral contractions in response to vagal stimulus. Taking into account that gastric emptying rate depends mainly on the antral activity, the gastric content would be released with higher frequency to the duodenum under the effect of nicotine. Furthermore, significant smoke-related differences observed in both formulations for AUC_{inf} would reveal some effect that tobacco could have on the intestinal transit time as well. Nicotine increases the intestinal peristalsis, and as CyA is absorbed at the first portion of the small intestine, an increase in the intestinal transit rate might not allow the drug to pursuit its complete absorption due to a major P-gp expression in the distal gut.

Interestingly, a significant smoke-related increase in C_{MAX}/AUC_{inf} was found when the Reference was the formulation given to the individuals, even though corrected C_{MAX} did not show any change. In order to facilitate the analysis of this phenomenon a one-compartment model with first-order input rate was considered. Any interruption in the process of drug

entrance leads to an increased C_{MAX}/AUC_{inf} value, as it is demonstrated throughout Equations 2 to 6,

Blood drug concentration (C) at each time (t) after oral administration can be described by Equation 2:

$$C(t) = \frac{[e^{-k_{EL}.t} - e^{-k_A.t}] . k_A . D}{[V_d . (k_A - k_{EL})]} \quad \text{(Equation 2)}$$

Where k_A: first order absorption rate constant; k_{EL}: first order elimination rate constant; D: absorbed dose; V_d: volume of distribution.

If the absorption process is interrupted at time T ($\leq T_{MAX}$), C(T) will become then the maximum blood concentration (Equations 3 and 4).

$$C(T) = \frac{[e^{-k_{EL}.T} - e^{-k_A.T}] . k_A . D}{[V_d . (k_A - k_{EL})]} \quad \text{(Equation 3)}$$

$$C(T) = \frac{e^{-k_{EL}.T}.[1 - e^{-(k_A - k_{EL}).T}] . k_A . D}{[V_d . (k_A - k_{EL})]} \quad \text{(Equation 4)}$$

The dose absorbed at time T is given by Equation 5:

$$D = [1 - e^{-k_A.T}] . D^* \quad \text{(Equation 5)}$$

where, D^*: administered dose.

The area under the curve decreases in relation with the case of non-interrupted absorption, as described by Equation 6:

$$AUC_{inf} = \frac{\left[1 - e^{-k_A \cdot T}\right] \cdot D^*}{V_d \cdot k_{EL}} < \frac{D^*}{V_d \cdot k_{EL}} \qquad \text{(Equation 6)}$$

Since $e^{-k_{EL} \cdot T}$ increases when T decreases (Equation 4), and $\left[1 - e^{-k_A \cdot t}\right]$ decreases to an extent greater than $\left[1 - e^{-(k_A - k_{EL}) \cdot T}\right]$ when T decreases (Equation 6 and 4, respectively), $\frac{C(T)}{AUC_{inf}}$ enhances its value. So, as it was deduced from Equations 2 to 6, any interruption happened at time $T \leq T_{MAX}$ provokes an increase in $\frac{C_{MAX}}{AUC_{inf}}$.

In accordance to this, smokers receiving the Reference formulation showed a lower AUC_{inf} and a higher C_{MAX}/AUC_{inf}. As the Reference formulation has an immediate CyA release into the stomach, a faster gastric emptying promoted by nicotine (Ferreira et al., 2002; Grimes and Goddard, 1987; Sanaka et al., 2005; Hanson and Lilja, 1987; Graff et al., 2001; Lagrue et al., 2006) would enhance the absorption rate to a magnitude that no difference in C_{MAX} could be observed even though an incomplete absorption of CyA would have taken place because of its rapid intestinal transit caused by nicotine (Graff et al., 2001; Lagrue et al., 2006) [see C_{MAX}* in Table V].

After administration of the Test formulation significant smoke-related differences were found in AUC_{inf} and C_{MAX} but not in C_{MAX}/AUC_{inf} (Table V). This could be explained by the fact that the Test formulation would release the drug slowly in the stomach, and hence, the absorption rate is now governed by the release from the formulation which is still at the stomach. The

absorption rate remained unchanged regardless the increase in gastric emptying observed in smokers, and thereafter no change in C_{MAX}/AUC_{inf} could be foreseen, since the interruption of drug absorption would be achieved when all CyA emulsion was released from the capsule. Nevertheless, a significant reduction in AUC_{inf} was found for both formulations in smokers (Table V), since the same fraction of drug molecules already in the duodenum does not have the time to be absorbed due to an increased intestinal peristalsis.

Conclusion

Sex- and smoke-related differences in the gastrointestinal transit could be detected in humans by the use of soft capsules of CyA microemulsion (Sandimmun Neoral ®-Novartis). This drug and formulation were able to reveal such differences because of two major characteristics: 1) drug absorption is restricted to the upper zone of the intestine; and 2) the immediate release of capsules content into the stomach. A generic formulation (Sigmasporin Microral ®) was not able to distinguish between male and female, or smokers and non-smokers, because CyA transit throughout the gastrointestinal tract was controlled by the formulation instead of the physiological status of the individuals.

Conflicts of interest

All authors declare that they have no conflicts of interest concerning this work.

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Figure captions

Figure 1. Mean CyA blood concentration (±standard errors) versus time profiles in male (n=8).

Figure 2. Mean CyA blood concentration (±standard errors) versus time profiles in female (n=8)

Figure 3. Mean CyA blood concentration (±standard errors) versus time profiles for smokers and non-smokers.

Figure1

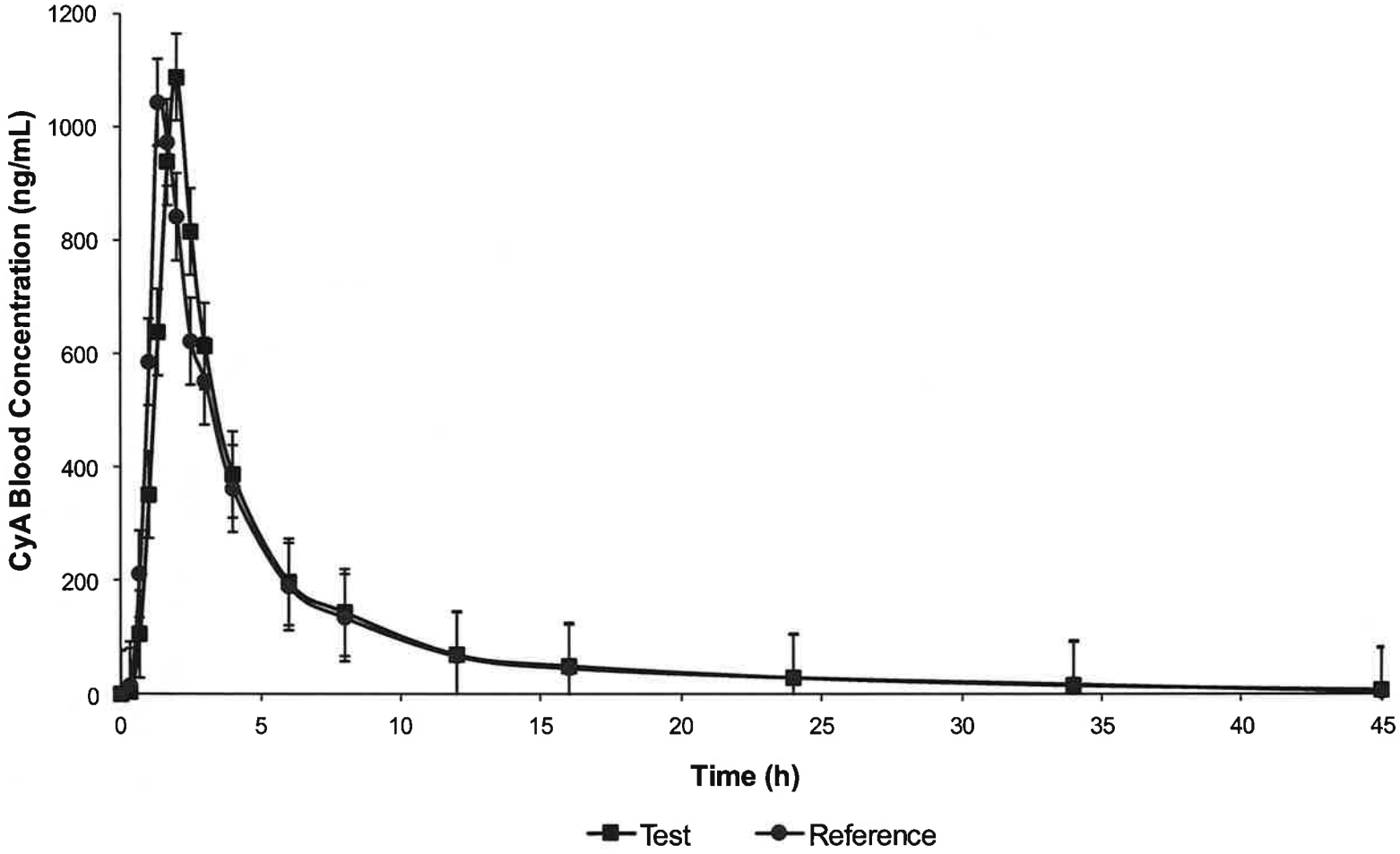

CyA Blood Concentration (ng/mL)
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
35
40
45
Time (h)
Test
Reference

Figure2

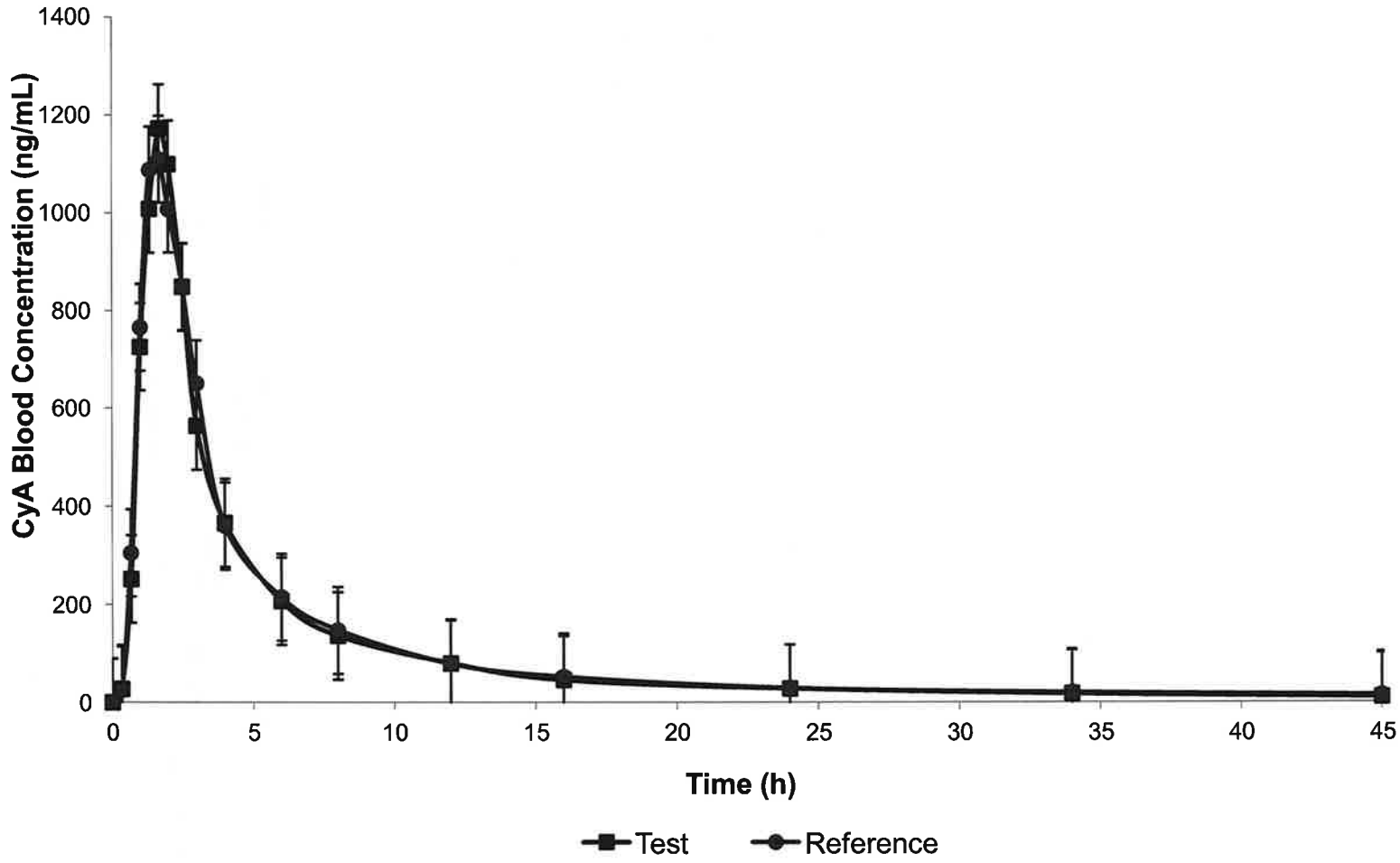
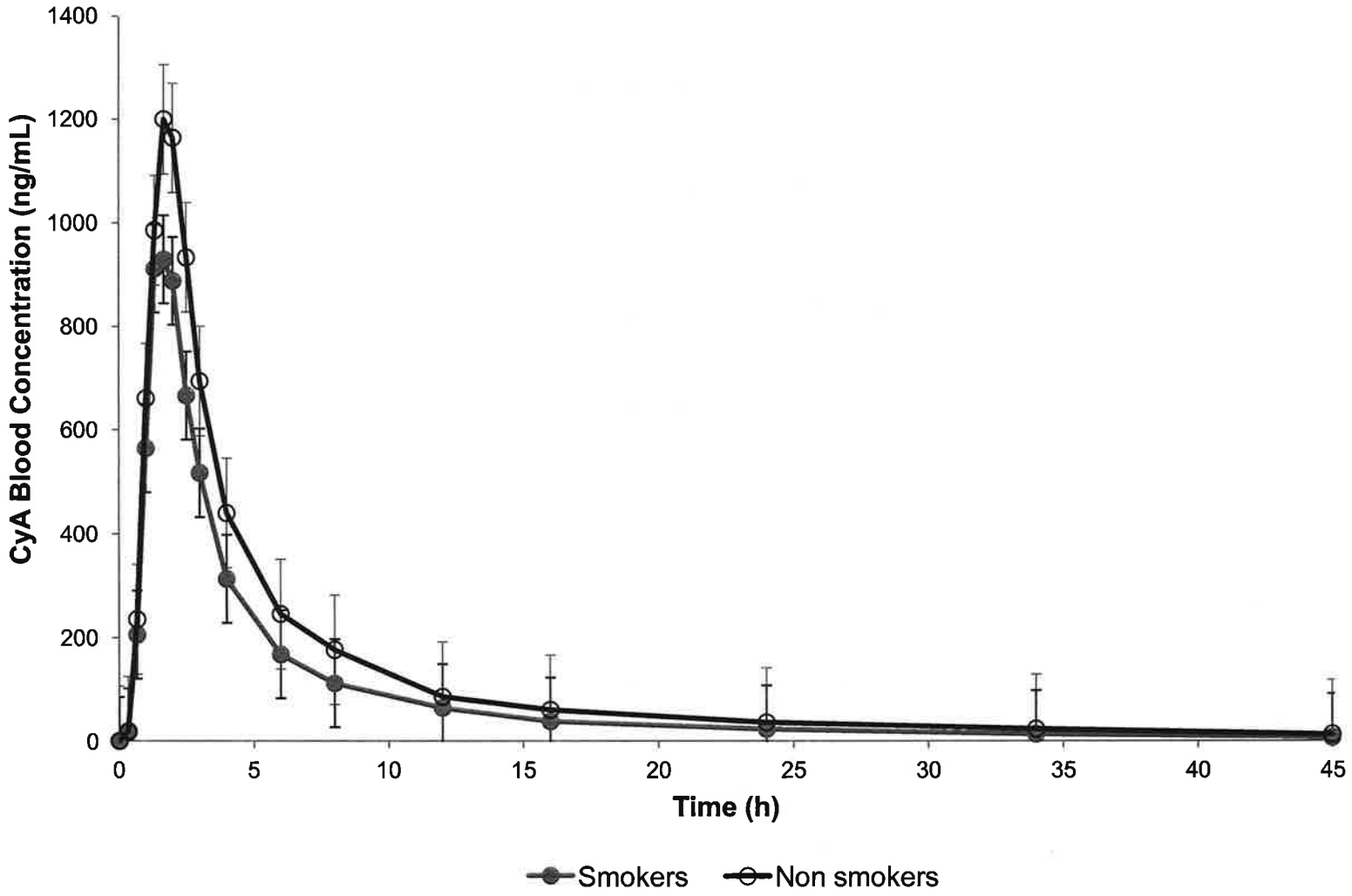

CyA Blood Concentration (ng/mL)
1400
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
35
40
45
Time (h)
Test
Reference

Figure3


1400
1200
1000
800
600
400
200
0
CyA Blood Concentration (ng/mL)
0
5
10
15
20
25
30
35
40
45
Time (h)
Smokers
Non smokers

Tables I to V

Table I. Pharmacokinetic parameters for CyA obtained after 200 mg of Test (T) or Reference (R) dose administration in 16 healthy subjects

Subject	T_{MAX} (h)		C_{MAX} (ng/mL)		AUC_T (ng. h/mL)		AUC_{inf} (ng. h/mL)		$T_{1/2}$ (h)	
Number	T	R	T	R	T	R	T	R	T	R
Male										
1[a]	2.00	1.33	847.8	858.6	3329	2956	3774	3235	22.2	18.5
2[a]	2.00	1.67	1250	1619	3958	5071	4213	5136	15.1	8.43
3	2.00	1.33	1524	1415	5655	6557	5833	6947	11.2	14.2
4	2.00	1.33	1367	1250	6043	5187	6176	5971	9.64	24.4
5	2.00	1.67	993.3	790.8	4681	4298	4954	4411	14.8	10.5
6[a]	2.00	1.33	746.8	792.6	3182	3049	3198	3130	6.89	11.5
7[a]	2.00	1.33	956.1	903.0	4416	3669	4782	3793	16.2	11.6
8	2.00	1.33	1022	945.3	4392	4286	4764	4730	17.7	18.1
Mean[b]	2.00	1.33	1088	1072	4457	4384	4712	4669	14.2	14.7
SD[c]	2.00-2.00	1.33-1.67	267.0	315.3	1011	1206	991.9	1329	4.85	5.31
Female										
9	1.67	2.00	2064	1335	7140	6335	7702	6640	18.2	13.8
10[a]	1.67	1.33	807.3	1013	3691	4112	3793	4800	10.9	26.2
11[a]	1.33	1.67	966.6	1029	3030	2989	3041	3028	6.06	9.24
12[a]	1.33	1.33	986.7	1215	4865	5083	4946	5369	9.27	14.8
13[a]	2.00	1.33	945.0	766.9	3881	3068	4411	3553	23.6	23.8
14	1.67	1.33	2288	1863	6236	7840	6452	8362	11.7	15.1
15	1.67	2.00	967.5	1147	4564	4493	5226	4870	21.7	18.1
16[a]	2.00	1.67	1353	1628	5249	6299	5460	6549	13.0	12.4
Mean[b]	1.67	1.50	1297	1250	4832	5027	5129	5396	14.3	16.7
SD[c]	1.33-2.00	1.33-2.00	567.1	353.8	1363	1705	1473	1745	6.21	5.75
Total										
Mean[b]	2.00	1.33	1193	1161	4645	4706	4920	5033	14.3	15.7
SD[c]	1.33-2.00	1.33-2.00	441.6	336.5	1175	1465	1232	1545	5.38	5.45

[a] Smoking subjects; [b] Median is informed for T_{MAX}; [c] Range, instead of standard deviation (SD), is informed for T_{MAX}.

Table II. Test (T) and Reference (R) geometric means of pharmacokinetic parameters, with their respective 90% confidence interval (90%CI) of the T/R ratios and ANOVA coefficient of variations (CV), considering only male subjects (n=8).

Parameters	Test Mean	Reference Mean	T/R	IC 90%		CV (%)
AUC_T (ng.h/mL)	4358	4243	1.03	0.93	1.13	10.08
AUC_{Inf} (ng.h/mL)	4557	4338	1.05	0.93	1.19	12.99
C_{MAX} ng/mL	1060	1035	1.02	0.94	1.12	9.154
C_{MAX}/AUC_{Inf}	0.2297	0.2295	1.00	0.90	1.11	11.15

Table III. Test (T) and Reference (R) geometric means of pharmacokinetic parameters, with their respective 90% confidence interval (90%CI) of the T/R ratios and ANOVA coefficient of variations (CV), considering only female subjects (n=8).

Parameters	Test Mean	Reference Mean	T/R	IC 90%		CV (%)
AUC_T (ng.h/mL)	4667	4773	0.98	0.90	1.06	8.112
AUC_{inf} (ng.h/mL)	4940	5166	0.96	0.86	1.06	10.93
C_{MAX} ng/mL	1207	1207	1.00	0.84	1.19	18.46
C_{MAX}/AUC_{inf}	0.2441	0.2346	1.04	0.88	1.22	16.82

Table IV. Test (T) and Reference (R) geometric means of pharmacokinetic parameters, with their respective 90% confidence interval (90%CI) of the T/R ratios and ANOVA coefficient of variations (CV) considering all the 16 subjects.

Parameters	Test Mean	Reference Mean	T/R	IC 90%		CV (%)
AUC_T (ng.h/mL)	4510	4500	1.00	0.95	1.06	8.77
AUC_{Inf} (ng.h/mL)	4745	4734	1.00	0.93	1.08	12.01
C_{MAX} ng/mL	1131	1118	1.01	0.93	1.10	13.49
C_{MAX}/AUC_{Inf}	0.2368	0.2321	1.02	0.94	1.11	13.61

Table V. Pharmacokinetic parameters and statistical comparisons between smokers (SMK) and non-smokers (NSMK). Significant differences were assessed when $p \leq 0.05$

Parameter	Formulation	SMK (n=9)	NSMK (n=7)	SMK / NSMK ratio	p-value
AUC_{inf} (ng.h/mL)	Test	4134	5869	0.7044	0.0024
	Reference	4257	5868	0.7254	0.0375
AUC_{inf}[a] (ng.h.kg/mL)	Test	298637	413935	0.7215	0.0088
	Reference	304604	410692	0.7417	0.0285
C_{MAX}[a] (ng/mL)	Test	71825	102999	0.6973	0.0445
	Reference	79312	87520	0.9062	0.5103
C_{MAX}/AUC_{inf} (h^{-1})	Test	0.2416	0.2439	0.9906	0.9291
	Reference	0.2611	0.2122	1.231	0.0242
k_{EL} (h^{-1})	Test	0.06176	0.04982	1.240	0.3502
	Reference	0.05260	0.04511	1.166	0.3861

(*) Weight corrected parameter