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ORIGINAL RESEARCH ARTICLE

Dose Escalation Study to Assess the Pharmacokinetic Parameters of a Nano-amorphous Oral Sirolimus Formulation in Healthy Volunteers

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Abstract

Background and Objectives Sirolimus (Rapamune®) exhibits low bioavailability, high variability and moderate food effect following oral administration. This makes therapeutic blood monitoring of sirolimus concentrations necessary for kidney transplant patients. Furthermore, reaching therapeutic blood sirolimus concentrations in renal cancer patients was found to be challenging when the marketed drug was administered alone. A novel, nano-amorphous formulation of the compound was developed and its pharmacokinetic properties were investigated in a dose escalation study in a first-in-human clinical trial. The effect of food at the highest dose on the pharmacokinetic parameters was also assessed.

Methods Each group received one of the escalating doses (0.5–2–10–40 mg) of sirolimus as the novel formulation in the fasted state. Following a 2- to 3-week washout period, the 40-mg group then also received another 40 mg dose in the fed state. Sirolimus whole blood concentrations were determined for up to 48 h. To avoid degradation of sirolimus in the acidic environment in the stomach, 40 mg famotidine was administered 3 h pre-dose in all regimens. The main pharmacokinetic parameters were calculated and data were compared with pharmacokinetic data reported for dose escalation studies for Rapamune®.

Results Thirty-two healthy volunteers were divided into 4 cohorts of 8 volunteers. Dose increments resulted in approximately dose-proportional increases of maximal plasma concentrations (C_{max}) and area under the concentration–time curve $(AUC)_{0-48\ h}$ up to 10 mg, while less than dose-proportional increases were observed when the dose was increased from 10 to 40 mg. Mean AUC_{inf} at the 40 mg dose in the fasted state was 4,300 ± 1,083 ng·h/ml, which is 28% higher than the AUC reported following the administration of 90 (2 × 45) mg Rapamune® and 11% higher than the exposure reported for 25 mg intravenous pro-drug temsirolimus (3,810 ng·h/ml). At the 40 mg dose, food reduced C_{max} by 35.5%, but it had no statistically significant effect on AUC. Inter-individual variability of the pharmacokinetic parameters mostly fell in the 20–30% (CV) range showing that sirolimus administered as the nano-amorphous formulation is a low-to-moderate variability drug.

Conclusion Based on the pharmacokinetic profiles observed, the nano-amorphous formulation could be a better alternative to Rapamune® for the treatment of mammalian target of rapamycin-responsive malignancies. Therapeutically relevant plasma concentrations and exposures can be achieved by a single 40 mg oral dose. Furthermore, the low variability observed might make therapeutic blood monitoring unnecessary for transplant patients taking sirolimus as an immunosuppressant.

Key Points

The nano-amorphous formulation could be a potential oral alternative to intravenous Torisel® for the treatment of mammalian target of rapamycin-responsive malignancies.

Low inter-individual variability might make therapeutic drug monitoring unnecessary for patients taking sirolimus.

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1 Introduction

Sirolimus (rapamycin) is a macrocyclic lactone which acts via the inhibition of the mammalian target of rapamycin (mTOR). It was developed as an immunosuppressant which is administered after kidney and liver transplantations [1, 2]. Later, mTOR was described as an atypical protein kinase that controls growth and metabolism in response to nutrients, growth factors and cellular energy levels frequently dysregulated in cancer and metabolic disorders (reviewed in [3]). The anti-proliferative effect of sirolimus and its analogs has been shown in various types of cancers and cancer models (reviewed in [4–6]). Analogs of sirolimus like everolimus and its ester prodrug temsirolimus (Torisel®) have already been approved for the treatment of advanced renal cell carcinoma. Everolimus has also been approved for the treatment of hormone receptor-positive advanced breast cancer, neuroendocrine tumors of pancreatic, gastrointestinal or lung origin and subependymal giant cell astrocytoma.

While sirolimus was shown to possess many therapeutically advantageous effects, oral delivery of the drug is challenging due to its unfavorable dissolution characteristics, stability issues in the gastrointestinal tract, participation of the molecule in multiple active processes and interaction with food. The compound is insoluble in aqueous media across the biorelevant pH range [7]. In order to improve dissolution an oral solution was developed (1 mg/ml Rapamune® oral solution) initially. Later, a wet-milled nanocrystal formula was introduced which allowed the development of a tablet form (0.5, 1 and 2 mg Rapamune® tablet). In healthy subjects, the mean bioavailability of sirolimus after administration of the tablet is approximately 27% higher relative to the solution [8]. Sirolimus tablets are not bioequivalent to the solution; however, clinical equivalence has been demonstrated at the 2 mg dose level [9]. Even when using these advanced formulations, the bioavailability of sirolimus remains low and highly variable [10]. In stable renal transplant patients, the apparent oral bioavailability of Rapamune® oral solution has been estimated to be about 15% [11]. Sirolimus also shows extremely rapid degradation in acidic solutions [12]; the half-life of the molecule was reported to be 5 min at pH 1.2, indicating practically immediate decomposition in the fasted state stomach [13]. Sirolimus exhibits extensive intestinal and hepatic first-pass metabolism by the CYP3A4 isoenzyme and counter transport by intestinal PgP [14]. These active processes were implicated as the primary source of the highly variable nature of sirolimus pharmacokinetics [15]. In food effect studies, a high fat meal decreased sirolimus area under the concentration–time curve (AUC) by 35% for Rapamune® oral solution when compared to the fasted state [7], while in healthy volunteers receiving Rapamune® tablets with a high-fat meal, maximal plasma concentration (C_{max}), time to reach C_{max} (t_{max}) and AUC increased by 65, 32 and 23%, respectively. The effect of food on the mean sirolimus C_{max} was inconsistent depending on the Rapamune® dosage form evaluated [8].

Dose escalation studies with orally administered Rapamune® tablets alone or in combination with pharmacokinetic modulators in advanced renal cell carcinoma cancer patients have already been conducted [16, 17]. The aim of the dose escalation was to achieve the target AUC of 3,810 ng·h/ml [16], i.e., the sirolimus exposure achieved by 25 mg Torisel®. The authors concluded that the recommended doses are 90, 16, and 35 mg when administered alone, with ketoconazole, and with grapefruit juice, respectively. When sirolimus was administered alone at high doses (60–90 mg), gastrointestinal toxicity necessitated splitting the dose into two equal administrations. The authors also noted that there are challenges to incorporating grapefruit juice, ketoconazole, or any inhibitor of drug metabolism into regular clinical practice [16]. These observations show that reaching therapeutic sirolimus blood concentrations necessary for the treatment of mTOR-responsive malignancies by the administration of oral Rapamune® remains a challenge.

Previously, we have developed and characterized a nano-amorphous formulation of sirolimus through in vitro and preclinical in vivo experiments. The formulation exhibited higher solubility, higher apparent permeability and faster and more complete absorption when compared to Rapamune® [18]. The aim of this first-in-human investigation was to characterize the clinical pharmacokinetic properties of this formulation in a dose escalation study and to assess the effect of food on the pharmacokinetic parameters.

2 Subjects and Methods

2.1 Preparation of the Investigational Medicinal Product

The nano-amorphous sirolimus formulation was produced by controlled precipitation followed by freeze drying. A methanolic solution was prepared containing 10 mg/ml and 30 mg/ml sirolimus (Concord Biotech Ltd., India) and polyvinylpyrrolidone 90F (BASF, Ludwigshafen, Germany), respectively. A second solution containing 5 mg/ml sodium lauryl sulfate (BASF) in water was also prepared. The two solutions were mixed together well using 1,500 rpm stirring at room temperature. During the solvent mixture preparation a 1:4 methanol:water ratio was used. Aliquots of the

resulting liquid were dispensed into glass containers and samples were immediately frozen on dry ice. The samples were freeze dried for 36 h using a ScanVac CoolSafe freeze drier (LaboGene Aps, Allerød, Denmark) with a − 110 °C ice condenser equipped with a Vacuubrand RZ6 vacuum pump. The investigational medicinal product (IMP) was a powder in a bottle (PiB) formulation containing 10 mg sirolimus for reconstitution with water. No residual methanol was detected in the IMP.

2.2 Study Population

Healthy subjects were selected by the investigators based on their medical history, physical examination, electrocardiograms and routine clinical laboratory test results. All subjects gave written informed consent and received an inconvenience allowance for their participation.

2.3 Clinical Study Design

The study was conducted at Quotient Sciences (Nottingham, UK) in accordance with the Clinical Protocol, with the Declaration of Helsinki and its amendments, with the International Conference on Harmonisation Good Clinical Practice (ICH GCP) Guidelines, and in accordance with all applicable regulatory requirements (EudraCT number: 2016-005018-23). This was a single center, open-label, non-randomized, single-dose study in healthy subjects to assess the safety and pharmacokinetics of single ascending doses of a novel sirolimus formulation, and to assess food effect. The low doses were selected based on the currently used Rapamune® tablet dosage strengths (0.5 and 2 mg). The decision to proceed to the next higher dose level was based on safety, tolerability and available pharmacokinetic data. The following data were required—adverse events (AEs), vital signs, safety laboratory parameters, physical examinations, whole blood concentrations of sirolimus, and interim pharmacokinetic parameter estimations for sirolimus (C_{max}, C_{max}/D, t_{max}, AUC and AUC/D). Decisions were made after a complete review of all data collected from the previous dose group by a Safety Advisory Committee, comprising the investigator, the sponsor's medical representative monitor and a pharmacokinetics expert where appropriate. Four cohorts of 8 subjects (32 subjects in total) across a total of 5 study periods were enrolled, to ensure 6 evaluable subjects per cohort. An evaluable subject was defined as a subject who had completed the planned safety and pharmacokinetic assessments up to 24 h after dosing. For the food effect study, the same cohort of subjects received a 40-mg formulation as a 2-period crossover to allow fed/fasted comparisons to be made at equivalent doses. The wash-out period was 2–3 week between administrations according to the half-life of sirolimus [7]; an evaluable subject was defined as a subject who had received the relevant dose in both the fed and fasted state.

The IMP used to support the treatment of subjects was prepared as a 10 mg unit dose PiB formulation. Doses < 10 mg were achieved by reconstituting the IMP using 50 ml of sterile water for irrigation, taking the appropriate aliquot to achieve the required dose strength and then making up to 50 ml with sterile water for irrigation. For doses > 10 mg, multiple bottles were used as needed to achieve the required dose. After oral administration, additional water was added to each bottle and dosed as a rinsing step to ensure the total dose was given. The total volume administered in all treatment periods was 240 ml.

As sirolimus stability is reduced by strongly acidic environments, 40 mg famotidine (histamine H2 receptor antagonist that inhibits gastric acid production) was administered 3 h pre-dose, in order to increase gastric pH.

Venous blood samples of approximately 4 ml were collected for the determination of blood concentrations (sirolimus is highly bound to red blood cells, therefore, it can only be properly quantitated from whole blood [reviewed in ref. 7]) immediately prior to dosing and at 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, and 48 h post dose. Following completion of each regimen, there was a period of interim analysis to determine the dose progression and the fed/fasted state to be used in the subsequent study period. For dose progression and fed/fasted state selection to proceed, data must have been available from a minimum of 6 evaluable subjects.

2.4 Bioanalytical Method

Human blood samples were analyzed for sirolimus using a validated liquid chromatography with tandem mass spectrometry method (based on [19]) at LGC Ltd (Fordham, Cambridge, UK). Method validation was based upon 'Guideline on Bioanalytical Method Validation', EMA, CHMP, EWP, July 2011 with reference to the 'Guidance for Industry, Bioanalytical Method Validation' recommendations issued by the U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), May 2001, BP.

The bioanalytical method was found to be linear for sirolimus over the calibration range of 0.1–200 ng/ml. The limit of quantification was 0.1 ng/ml. All blood sirolimus concentrations below this threshold were considered zero. The precision and accuracy of the method was found to be within the target limits of within 20% at the lower limit of quantification and within 15% at all other concentrations. The recovery of sirolimus from human plasma was consistent

across the analytical range and acceptable and no significant matrix effects were observed.

2.5 Pharmacokinetic Evaluation

The plasma concentration time profile of sirolimus was analyzed using noncompartmental methods with the linear trapezoidal rule using the WinNonlin pharmacokinetics software (v6.3; Certara USA, Inc., USA).

Statistical analysis was performed on the C_{max} and AUC from time 0 to the last quantifiable concentration (AUC_{last}) for sirolimus to assess dose proportionality for all dose levels administered in the fasted state. This was performed on the entire dose range initially (i.e., 0.5–40 mg) and then as an exploratory analysis on a reduced dose range (i.e., 0.5–10 mg, excluding the 40 mg dose level). AUC form time 0 to infinity (AUC_{inf}) was calculated using elimination rate constants determined based on whole blood sirolimus concentrations at late time points.

To assess food effect, the C_{max} and AUC_{last} for sirolimus underwent a natural logarithmic transformation and were analyzed using mixed-effect modeling techniques. The model included terms for subject fitted as a random effect, and prandial state as a fixed effect. Adjusted geometric mean ratios and 90% confidence intervals (CI) for the adjusted geometric mean ratios were calculated. In addition, *p* values (for the null hypothesis of no food effect) and the intra-subject variability values (denoted as CV_w) were also presented.

2.6 Safety Evaluations

Safety was assessed in all subjects through monitoring of changes in vital sign values, clinical laboratory test results, physical examinations, electrocardiograms (ECGs) and adverse event reports. Any clinically significant abnormality in laboratory parameters, vital signs or ECGs could have been reported as an AE according to the judgement of the principal investigator (PI), taking into account any associated clinical signs and symptoms and pre-dose values. A serious AE was any untoward medical occurrence or effect that, at any dose, resulted in death, was life-threatening, required or prolonged inpatient hospitalization, resulted in persistent or significant disability/incapacity, was a congenital anomaly/birth defect or was an important medical event as recognized by the PI.

3 Results

3.1 Subject Disposition and Baseline Characteristics

The demographic and baseline characteristics of the volunteers are presented in Table 1. Overall, 32 subjects (27 males and 5 females) between 30 and 63 years of age were entered into the study. There were 30 white subjects and 2 Asian subjects enrolled with no current smokers. The majority had an alcohol consumption of between 1 and 14 units per week. Overall there were no notable differences in demographic variables between cohorts.

One subject reported taking medications prior to dosing—Subject 001 took 2 doses of 1 g paracetamol for toothache, 10 days prior to dosing. In addition, Subject 005 had an ongoing intrauterine contraceptive (Mirena® [levonorgestrel]) throughout the study.

Two subjects reported a pre-dose AE that were ongoing during dosing—Subject 005 had moderate dysmenorrhea, and Subject 019 had a mild wound (cut to left knee).

All subjects had negative alcohol breath test results, carbon monoxide (CO) breath test results of ≤ 10 ppm and negative virology and urine drug screen results, with the exception of Subject 026 (Cohort D) who had a CO breath test result of 11 ppm on admission to Period 1; a repeat test at an unscheduled time point gave a result of 9 ppm.

All female subjects had negative urine pregnancy test results at screening and admission, and all post-menopausal females had follicle-stimulating hormone test results ≥ 40 IU/l.

All 32 subjects received the IMP (nano-amorphous sirolimus formulation), had a minimum of 1 valid post-dose analytical result for pharmacokinetic parameter estimation, and were therefore included in both the pharmacokinetic and safety populations of the study.

3.2 Pharmacokinetics of Sirolimus

Following administration of the formulation, the mean sirolimus blood concentrations increased rapidly to peak and declined in a biphasic manner. Mean t_{max} occurred within 1 h regardless of the dose or prandial status (Fig. 1a, Table 2). Dose increments resulted in approximately dose proportional increases of C_{max} and AUC_{last} up to 10 mg, while less than dose proportional increases were observed at the 40 mg dose (Table 3). Food reduced C_{max} by 35.5%, but it had no statistically significant effect on exposure (Table 4). The calculated $t_{1/2}$ based on the last three data points (12–24–48 h) was 30–40 h; substantially lower than the $t_{1/2}$ reported for Rapamune® (62 ± 16 h, [7]). This indicates that the data points used were insufficient to determine the real $t_{1/2}$; therefore, the calculation of AUC_{inf} was not possible based on these plasma concentrations. For the subjects involved in the 40 mg/food effect group, quantifiable sirolimus blood concentrations were detected before administration of the second dose 2–3 weeks after administration of the first dose in the fasted state (Fig. 1b). These plasma concentrations were $< 1\%$ of the respective C_{max} values and were not considered to impact the integrity of the pharmacokinetic parameter

Table 1 Subject demography

Parameter	Dose	Cohort A 0.5 mg ($n=8$)	Cohort B 2 mg ($n=8$)	Cohort C 10 mg ($n=8$)	Cohort D 40 mg ($n=8$)	Overall ($n=32$)
Age (years)	Mean	52.3	50.5	55.6	49	51.8
	Median	50	54	55.5	48.5	52
	SD	6.6	10.8	4.1	7.1	7.6
	Minimum	43	30	51	40	30
	Maximum	61	62	63	63	63
Race (*n* %)	White	7 (87.5)	8 (100)	7 (87.5)	8 (100)	30 (93.8)
	Black	0	0	0	0	0
	Asian	1 (12.5)	0	1 (12.5)	0	5 (15.6)
	Other	0	0	0	0	0
Sex (*n* %)	Male	6 (75)	8 (100)	6 (75)	7 (87.5)	27 (84.4)
	Female	2 (25)	0	2 (25)	1 (12.5)	5 (15.6)
Height (cm)	Mean	169.5	177.8	174	174.5	173.9
	Median	170	180	176	176	175.5
	SD	7.1	5.8	10.4	6.7	7.9
	Min	160	169	153	159	153
	Max	180	186	186	180	186
Weight (kg)	Mean	79.75	87.25	86.95	86.18	85.03
	Median	76.5	84.3	81.6	88.9	84.3
	SD	8.66	7.94	13.9	8.85	10.11
	Min	70.2	77.5	74	69	69
	Max	93.2	100.9	109.7	94.4	109.7
Body mass index (kg/m^2)	Mean	27.75	27.64	28.68	28.24	28.08
	Median	27.45	27.2	26.95	28.35	27.7
	SD	2.39	2.41	3.43	1.54	2.44
	Min	24.7	24.5	25.1	25.6	24.5
	Max	31.1	31.1	34.4	30.5	34.4
Does subject smoke, use e-cigarettes or other nicotine products	Yes	0	0	0	0	0
	No	5 (62.5)	5 (62.5)	6 (75)	5 (62.5)	21 (65.5)
	Previously	3 (37.5)	3 (37.5)	2 (25)	3 (37.5)	11 (34.4)
Alcohol consumption	None	3 (37.5)	0	3 (37.5)	1 (12.5)	7 (21.9)
	1–14 units/week	5 (62.5)	7 (87.5)	3 (37.5)	6 (75)	21 (65.6)
	15–21 units/week	0	1 (12.5)	2 (25)	1 (12.5)	4 (12.5)

estimates in the fed state. At the same time, this allowed the determination of $t_{1/2}$ and the determination of AUC_{inf} at the 40 mg dose in the fasted state. The mean (± SD) $t_{1/2}$ value was 72.5 ± 8.7 h, while the mean AUC_{inf} (± SD) was 4,300 ± 1,083 ng·h/ml. The pharmacokinetic curves for the 8 volunteers were surprisingly tight for all doses and prandial states with a ~ 2-fold ratio observed between the highest and lowest C_{max} and AUC values (Table 2). Variability exhibited an apparent decreasing trend with increasing dose (Fig. 1c). Inter-individual variability of the pharmacokinetic parameters mostly fell in the 20–30 CV % range showing that sirolimus is a low-to-moderate variability drug when administered as the novel formulation (Table 2).

3.3 Safety

The sirolimus PiB formulation was well tolerated under the conditions of this study; no deaths or severe AEs were reported, and no subject was withdrawn as a result of an AE (Table 5). All AEs reported in the study were mild in severity, with the exception of one moderate mouth ulceration reported following dosing with 40 mg sirolimus in the fasted state. The majority of AEs were unrelated to the IMP. Three (9.4%) subjects reported a total of 4 IMP-related AEs, comprising oral herpes (10 mg fasted), mouth ulceration and headache (40 mg fasted), and increased alanine aminotransaminase (ALT) (40 mg fed).

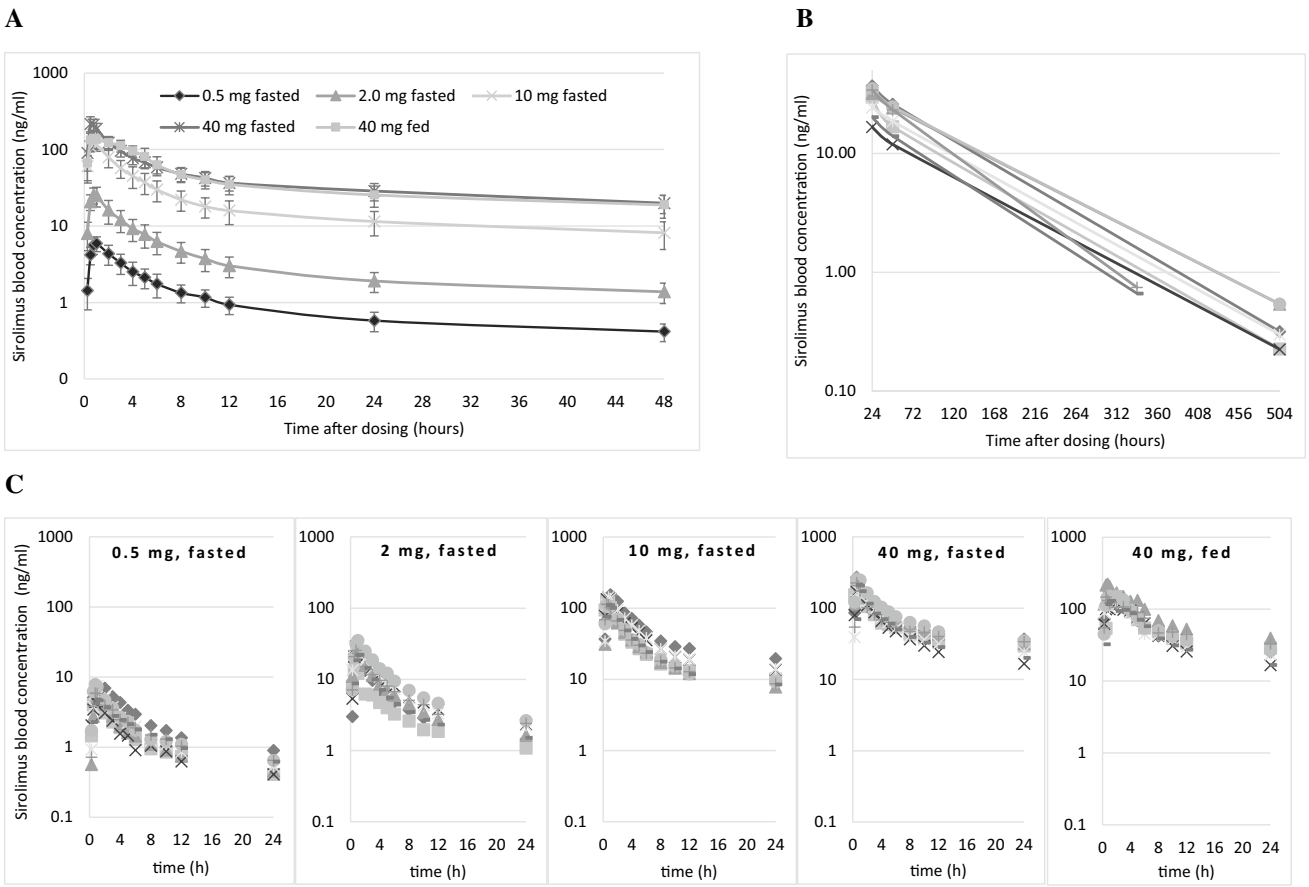

Fig. 1 **a** Mean (±standard deviation) blood sirolimus concentrations in the first 48 h following administration of the novel formulation at the indicated dose and prandial state. **b** Individual blood sirolimus concentrations at late time points (used for the calculation of area under the concentration–time curve from time 0 to infinity (AUC_{inf}) for the 40 mg dose in the fasted state (**c**). Individual blood sirolimus concentrations in the first 24 h at the indicated doses and prandial states

The mouth ulcers were possibly related to the immunosuppressant nature of the IMP. The subject with elevated ALT 6 days post dose resolved spontaneously in 8 days. Other than that, no subject had clinically significant laboratory parameters, vital signs or ECG results.

4 Discussion

In agreement with our previously reported in vitro and preclinical data [18] the nano-amorphous formulation of sirolimus exhibited markedly improved pharmacokinetics when compared to Rapamune®. C_{max} values were 3–5 times higher when compared to the values reported for dose escalation studies of Rapamune® when administered alone and were in the range or even exceeded the ones observed with the co-administration of metabolic and transport inhibitors [16]. Mean AUC_{inf} at the 40 mg dose (4,300 ng·h/ml) was 28% higher than the AUC_{inf} reported following the administration of 90 (2×45) mg Rapamune® when administered alone (3,356 ng·h/ml, [16]), while it was 11% higher than the AUC reported for 25 mg intravenous Torisel® (3,810 ng·h/ml, [16]).

The formulation was designed to improve sirolimus solubility thereby making sirolimus more readily available for absorption. This resulted in strikingly tight data across the 8 volunteers in all 5 administrations indicating a substantial improvement in the inter-individual variability when compared to Rapamune®. This indicates that the dissolution and absorption process still carry a large portion of the observed variability of Rapamune®, while other factors (CYP3A4, PgP) play a lesser role than previously suggested. Furthermore, from a therapeutic perspective the low inter-individual variability observed might make therapeutic drug monitoring unnecessary in transplant patients.

As sirolimus stability is reduced by a strongly acidic environment 40 mg famotidine (histamine H2 receptor antagonist that inhibits stomach acid production) was administered

Table 2 Pharmacokinetic parameters of sirolimus following administration of the formulation at the indicated doses and prandial states

Statistic	t_{max} (h)	C_{max} (ng/ml)	C_{max}/D (ng/ml/mg)	C_{24} (ng/ml)	$AUC_{(0-24)}$ (ng·h/ml)	AUC_{last} (ng·h/ml)	AUC_{last}/D (ng·h/ml/mg)	$t^{a}_{1/2}$ (h)
0.5 mg fasted ($n=8$)								
Median	1	5.67	11.3	0.579	36.8	48.9	97.7	33.47
Min	0.75	4.53	9.06	0.41	25.9	34.6	69.2	25.75
Max	1	7.92	15.8	0.904	55.3	73.7	147	37.18
Geometric mean	NC	5.91	11.8	0.561	35.5	47.1	94.3	30.90
Geometric CV %	NC	22.9	22.9	27.9	24.8	25.1	25.1	16.50
2 mg fasted ($n=8$)								
Median	0.75	26.7	13.3	1.95	134	175	87.7	29.39
Min	0.75	13.7	6.85	1.08	70.7	92.5	46.2	25.26
Max	1	35.1	17.6	2.63	194	248	124	39.12
Geometric mean	NC	25.1	12.5	1.83	128	166	82.8	30.56
Geometric CV %	NC	30	30	32.2	32.5	32.3	32.3	18.30
10 mg fasted ($n=8$)								
Median	0.71	121	12.1	9.63	566	762	76.2	36.25
Min	0.5	96.6	9.66	7.84	517	674	67.4	31.29
Max	1	155	15.5	19.6	1,010	1,420	142	40.77
Geometric mean	NC	124	12.4	10.9	652	877	87.7	36.51
Geometric CV %	NC	16.5	16.5	32.9	24.6	27	27	8.80
40 mg fasted ($n=8$)								
Median	0.5	221	5.51	30.9	1,250	1,880	46.9	73.35
Min	0.5	174	4.35	16.7	999	1,340	33.5	57.14
Max	0.75	275	6.88	37	1,690	2,400	60.1	82.25
Geometric mean	NC	219	5.48	27.7	1,290	1,860	46.4	72.02
Geometric CV %	NC	18.9	18.9	29.4	18.3	20.8	20.8	12.03
40 mg fed ($n=8$)								
Median	0.75	140	3.49	24.4	1,140	1,640	41	42.04
Min	0.5	111	2.78	16.7	1,030	1,370	34.3	27.91
Max	2	224	5.6	39.6	1,870	2,710	67.7	51.72
Geometric mean	NC	141	3.53	24.5	1,220	1,740	43.4	39.86
Geometric CV %	NC	23	23	30.7	20.4	23.1	23.1	19.50

NC not calculated

t_{max} time to maximal blood concentration, C_{max} maximal blood concentration, C_{max}/D dose corrected maximal blood concentration, C_{24} blood concentration at 24 h following administration, *AUC* area under the concentration–time curve, *AUC/D* dose corrected AUC, $t_{1/2}$ apparent half-life

*Calculated from 12 to 48 h except for 40 mg fasted (from 12 to 504 h)

3 h pre-dose to increase gastric pH in this study. Famotidine exhibits essentially no known drug–drug interactions with substrates of CYP3A4 or PgP which makes it an ideal H2 receptor antagonist to control the pH of the stomach without interference with sirolimus pharmacokinetics [20].

The current study is a first-in-human pharmacokinetic investigation using a novel nano-amorphous oral formulation of sirolimus. In comparison with published Rapamune® data the nano-amorphous formulation delivers pharmacokinetic advantages; however, a direct comparison of the formulation with Rapamune® would be necessary to obtain quantifiable data on the improvements. Another study should also be performed using an acid-resistant final dosage form to replace the H2 receptor agonist administration used in this study. Moreover, more precise determination of pharmacokinetic parameters would be possible by sampling at later time points following administration.

5 Conclusions

Based on the pharmacokinetic profiles observed, the oral administration of the nano-amorphous formulation of sirolimus could potentially be used as an alternative to

Table 3 Statistical analysis of dose proportionality of C_{max} and AUC_{last} in the fasted state

Parameter	Statistic	0.5 mg ($n=8$)	2 mg ($n=8$)	10 mg ($n=8$)	40 mg ($n=8$)
C_{max} (ng/ml)	Geometric mean	5.91	25.1	124	219
	$\beta_{0.5–10\ mg}$ (90% CI)	1.01 (0.95, 1.08)			
	$2\hat{}\beta_{0.5–10\ mg}$ (90% CI)	2.02 (1.93, 2.11)			
	$\beta_{0.5–40\ mg}$ (90% CI)		0.84 (0.79, 0.90)		
	$2\hat{}\beta_{0.5–40\ mg}$ (90% CI)		1.80 (1.72, 1.87)		
AUC_{last} (ng·h/ml)	Geometric mean	47.1	166	877	1860
	$\beta_{0.5–10\ mg}$ (90% CI)	0.98 (0.90, 1.06)			
	$2\hat{}\beta_{0.5–10\ mg}$ (90% CI)	1.97 (1.86, 2.08)			
	$\beta_{0.5–40\ mg}$ (90% CI)		0.86 (0.81, 0.92)		
	$2\hat{}\beta_{0.5–40\ mg}$ (90% CI)		1.82 (1.75, 1.89)		

n = total number of subjects in dose group. β (i.e., the slope parameter) and its 90% CI = a measure of dose proportionality. $2\hat{}\beta$ = the increase in exposure for a 2-fold increase in dose. Results obtained from log-transformed pharmacokinetic parameters using a power model. The model included a term for log dose fitted as a continuous covariate. Dose proportionality can be concluded if the 90% CI for β lies entirely within the critical region (0.93–1.07 for 0.5–10 mg and 0.95–1.05 for 0.5–40 mg)

C_{max} maximal blood concentration, *AUC* area under the concentration–time curve

Table 4 Statistical analysis of the food effect at the 40 mg dose ($n=8$)

Parameter	Fed adj geo mean (1)	Fasted adj geo mean (1)	Ratio (%) (2)	90% CI (3)	p value (4)	CVw (%) (5)
C_{max} (ng/ml)	141	219	64.46	56.29, 73.82	< 0.001	14.38
AUC_{last} (ng·h/ml)	1,740	1,860	93.63	84.35, 103.92	0.27	11.05

Results obtained from mixed-effect modelling analysis of natural log transformed pharmacokinetic parameters with terms for treatment fitted as a fixed effect and subject fitted as a random effect. (1) Adj geo mean = adjusted geometric mean from model; (2) ratio of adj geo mean (fed/fasted); (3) CI = confidence interval for ratio of adj geo means; (4) p value for null hypothesis of no treatment difference; (5) CVw = intra-subject (i.e., within subject) variability estimated from the model residual term

C_{max} maximal blood concentration, *AUC* area under the concentration–time curve

Table 5 Incidence of adverse events at the different study cohorts

System organ class preferred term	0.5 mg Fasted ($n=8$)	2 mg Fasted ($n=8$)	10 mg Fasted ($n=8$)	40 mg Fasted ($n=8$)	40 mg Fed ($n=8$)	Overall ($n=32$)
Subjects reporting TEAEs	0	1 (12.5)	1 (12.5)	4(50)	3 (37.5)	6 (18.8)
Infections and infestations	0	0	1 (12.5)	1 (12.5)	1 (12.5)	2 (6.3)
Folliculitis	0	0	0	1 (12.5)	1 (12.5)	1 (3.1)
Oral herpes	0	0	1 (12.5)	0	0	1 (3.1)
Nervous system disorders	0	1 (12.5)	0	1 (12.5)	0	2 (6.3)
Headache	0	1 (12.5)	0	1 (12.5)	0	2 (6.3)
Gastrointestinal disorders	0	0	0	1 (12.5)	0	1 (3.1)
Mouth ulceration	0	0	0	1 (12.5)	0	1 (3.1)
Injury poisoning and procedural complications	0	0	0	1 (12.5)	0	1 (3.1)
Ligament sprain	0	0	0	1 (12.5)	0	1 (3.1)
Investigations alanine aminotransferase	0	0	0	0	1 (12.5)	1 (3.1)
Increased	0	0	0	0	1 (12.5)	1 (3.1)
Musculoskeletal and connective tissue disorders	0	0	0	1 (12.5)	1 (12.5)	1 (3.1)
Muscle spasms	0	0	0	1 (12.5)	1 (12.5)	1 (3.1)
Skin and subcutaneous tissue disorders	0	0	0	0	1 (12.5)	1 (3.1)
Pruritus	0	0	0	0	1 (12.5)	1 (3.1)
Rash	0	0	0	0	1 (12.5)	1 (3.1)

Values are expressed as *n* (%). The majority of AEs were unrelated to the IMP. AEs related or possibly related to the IMP are detailed in the text

TEAE treatment emergent adverse events, *IMP* investigational medicinal product

intravenous Torisel® for the treatment of mTOR-responsive malignancies. Therapeutically relevant plasma concentrations and exposures can be achieved by a single 40 mg oral dose with low inter-individual variability. The later improvement might make therapeutic drug monitoring unnecessary for transplant patients.

Compliance with Ethical Standards

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Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval This study was approved by the NHS/HSC Research Ethics Committee.

Informed consent Informed consent was obtained from all individual participants included in the study.

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