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Tolerability, pharmacokinetics and concentration-dependent hemodynamic effects of oral CF101, an A3 adenosine receptor agonist, in healthy young men

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Key words
healthy volunteers – CF101 – A3 adenosine receptor agonist – pharmacokinetics – hemodynamics – tolerance

Abstract. Objectives: To assess safety, tolerability, pharmacokinetics and hemodynamic effects of oral CF101, an A3 adenosine receptor (A3AR) agonist, in healthy men. Methods: One single and 1 repeated dose, parallel-group, ascending dose, double-blind and placebo-controlled study in normal volunteers. In the single dose study, n = 15 subjects received 1, 5 or 10 mg oral CF101; in each group 1 subject received placebo, the remainder active CF101. In the repeat-dose study, n = 28 subjects received repeated 12-hourly oral doses of CF101 (2, 3, 4 or 5 mg) for 7 days, in each group 2 subjects received placebo, the remainder active CF101. Test materials: Single-dose study: CF101 in 30% Cremophor RH40. Multiple-dose study: CF101 in 0.5% methylcellulose suspension. Both studies: the corresponding vehicles were used as placebos. Galenicals were prepared remotely from the clinical study site to ensure double-blind nature of the study. Results tolerability: Single doses up to 5 mg CF101 were safe and well-tolerated. However, the single dose of 10 mg CF101 was associated with flushing, tachycardia, nausea and vomiting, which were viewed as dose-limiting in normal volunteers. Single doses of CF101 (as well as the first of the multiple doses) were associated with increases in heart rate (8 – 24 beats/min after 5 mg and 18 – 55 beats/min after 10 mg). Multiple doses up to 4 mg 12-hourly for 7 days were safe and well-tolerated. However, the 5 mg multiple-dose group reported headache, drowsiness, hot flushes and dizziness on standing; this declined with dosing duration and was not dose-limiting in this study. Adverse events were commonest near t_{max}. Results pharmacokinetics: For oral CF101, the t_{max} was always 1 – 2 h post-dose and $t_{1/2}$ about 9 h, in both the single- and multiple-dose studies. For a single 5 mg dose (mean ± SD) C_{max} = 81.6 ± 23.6 ng/ml in the single dose study, and 63.6 ± 22.0 ng/ml after the first of the multiple doses; AUC_{inf} was 904.0 ± 221.9 ng.h/ml and 596.1 ± 196.6 ng.h/ml for the 2 studies, respectively. After 7 days of multiple dosing there was little change, and $AUC_{0\text{-}24h}$ = 601.0 ± 163.6 ng.h/ml. These pharmacokinetic parameters were linearly proportional to dose in the other treatment groups. Results pharmacodynamics: Increases in heart rate were related to plasma concentration and evident only in the upper range of concentrations observed. There were no changes on ECG monitoring beyond sinus tachycardia, and, in particular, no evidence of PR prolongation in any subject (n = 43). In comparison with single doses, this response was almost absent after 7 days of dosing. Leucocytosis (increases up to about 1.5×10^9/l after 5 and 10 mg) was similarly transient and reversible after multiple dosing. Conclusions: Single oral doses up to 5 mg CF101 and repeated doses up to 4 mg 12-hourly for 7 days were safe and well-tolerated. Multiple-dose CF101 pharmacokinetics were unchanged and predictable from single-dose estimates, and were linearly proportional to dose. Increases in heart rate and neutrophil count were reversible during multiple dosing and were not dose-limiting in the repeat dose study. CF101 warrants further study for its efficacy in treating human disease.

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Introduction

Adenosine is a ubiquitous purine nucleoside which is secreted extracellularly by metabolically active and stressed cells. Adenosine is an important regulatory molecule through its binding to at least 4 G-protein-associated cell surface receptors, currently classified A1, A2a, A2b and A3 [Linden 1994, Poulsen et al. 1998]. Almost all human

tissues express adenosine receptors of 1 or more classes, and this includes, in high density, various tumor cells [Merighi et al. 2001]. A1 and A3 receptor activation causes G-protein signal transduction leading to reduced activity of kinases PKB/Akt and PKA, and decreased formation of cAMP; this inhibits cell growth [Fishman et al. 2002].

Methyl 1-[N6-(3-iodobenzyl)-adenin-9-yl]-β-D-ibofuronamide (CF101 or IB-MECA; mw = 510.29 Daltons) is a new, orally active, adenosine receptor agonist with specific, submicromolar potency at the A3 receptor (Ki = 0.47 μM [Bar-Yehuda et al. 2002]). CF101 has cytostatic effects on various tumor cell types by arresting cell growth at G0/G1 phase of the cell cycle [Fishman et al. 2000]. In vivo, orally administered CF101 inhibits the development of tumors in syngeneic (melanoma, colon carcinoma) and xenograft (colon and prostate carcinoma) mouse models [Bar-Yehuda 2001, Fishman et al. 2003]. Interestingly, giving CF101 orally to mice stimulates the production of neutrophils via an increase in granulocyte colony stimulating factor (G-CSF) and, correspondingly, CF101 protects against cytotoxic-induced myelotoxicity [Bar-Yehuda et al. 2002, Fishman et al. 2000]. Oral CF101 also inhibits progression of colon carcinoma in nude mice, and stimulates neutrophil recovery after cytotoxic drug therapy in this strain [Bar-Yehuda 2001, Fishman et al. 2003].

Here, we report the results from 2 human volunteer studies (single and repeated dose), which were designed as the initial assessments of safety, tolerability, pharmacokinetics and hemodynamic effects of oral CF101.

Methods

Galenicals

Oral, aqueous solutions of CF101 were used. In the single-dose study, the vehicle was 30% Cremophor RH40 (BASF) and the vehicle-only, 1, 5, and 10 mg doses were matched for volume (100 ml) and appearance. In the multiple-dose study, the vehicle was 0.5% methylcellulose suspension (Methocel A4M Premium, The Dow Chemical Company) and the vehicle-only 2, 3, 4, and 5 mg were similarly matched. Subjects took the study medication, followed by 2 sequential, swallowed 50 ml rinses of water, for a total volume of 200 ml for each dose. The solutions and treatment coding were prepared individually by staff who were not associated with any other part of the studies, according to an independently prepared randomization code. Clinical staff knew which group was being studied in these ascending dose studies, but were otherwise masked as to active or placebo treatments.

Study participants

The protocols were approved by the Brent Medical Ethics Committee, Central Middlesex Hospital, London. All volunteers gave fully informed, written consent. We conducted both studies in full compliance with current applicable Good Clinical Practice guidelines, and, inasmuch as they apply to normal volunteer studies, the principles of the Declaration of Helsinki, as amended.

All subjects (n = 43) were healthy at screening, and had, in particular, no history of cardiovascular or hematological disease, splenectomy or splenomegaly, which could have confounded the pharmacodynamic parts of the study.

In the single-dose study, the mean (range) age, weight and height were 28.3 (20 – 40) years, 75.9 (63 – 98) kg, and 177.8 (167 – 188) cm, respectively. In the multiple-dose study, the mean (range) age, weight and height were 25.2 (18 – 45) years, 75.3 (56 – 99) kg and 178.0 (163 – 189) cm, respectively. All volunteers were of European ethnic origin, except for 2 subjects with Asian/Indian and 1 subject of Caucasian/Eastern-Asian heritage.

Subjects were screened within 21 days of first dose and attended the ward the evening before dosing. While subjects were resident on the ward, no alcoholic or caffeinated drinks, grapefruit or grapefruit juice, smoking or exercise were allowed.

Study designs

Both studies used double-blind, placebo-controlled, parallel-group, ascending dose designs. In the first study, 3 groups of n = 5 subjects (4 active : 1 placebo) received a sin-

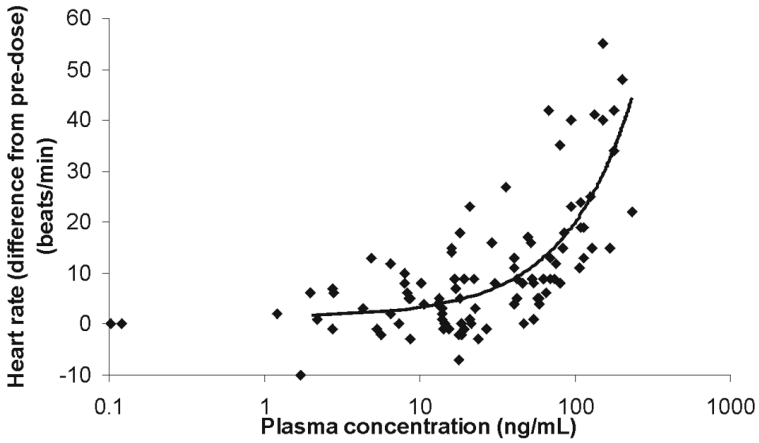

Figure 1. Concentration-dependency of semirecumbent heart rate after single doses of CF101 (0 – 24 h observation; n = 108 simultaneous pharmacokinetic and heart rate measurements in 12 active-treated normal subjects).

gle dose of oral CF101 (0, 1, 5 or 10 mg). In the second study, 4 groups of n = 7 subjects (5 active : 2 placebo) received oral doses of CF101 (0, 2, 3, 4 or 5 mg) every 12 h for 7 days.

Study procedures

The following procedures and safety parameters were used in both studies: history and physical examination, vital signs (semirecumbent in the single-dose study; semirecumbent and standing in the multiple-dose study), routine venous blood clinical biochemistry and hematology, urinalysis, 12-lead ECG, ambulatory ECG telemetry, pulmonary function testing (forced expiratory volume at time 1 sec: FEV_1). Adverse events were also recorded throughout both studies.

In the single-dose study, the following additional assessments were made: blood samples for CF101 pharmacokinetics pre dose and 0.25, 0.5, 1, 2, 4, 8, 12, 24, and 48 h after dosing; pharmacodynamic blood samples were taken for hematology (pre dose and 1, 2, 4, 8, 12, 24, 48, 72, 96, 120 and 144 h), G-CSF (pre dose and 1, 2, 4, 8, 12, 24, 48, 72 and 96 h), CD34+ cells (at pre-dose and 24, 48, 72, 96, 120 and 144 h post dose, and granulocyte-monocyte colony forming cells (GM-CFC; pre dose and 72, 96 and 120 h).

In the multiple-dose study blood samples for CF101 pharmacokinetics were taken on day 1 (pre dose and 0.25, 0.5, 1, 2, 4, 8, and 12 h), days 2 – 6 (just before the first dose each day, "trough values"), and day 7 (as for day 1). Pharmacodynamic samples for hematology were taken immediately before and 8 h after each morning dose on all 7 dosing days.

Pharmacokinetic and pharmacodynamic assays

Plasma samples were assayed for CF101 using a validated LC/MS/MS method. The lower limit of quantification (LLQ) was 0.1 ng/ml. Intraassay coefficient of variation (CV) was < 5.0% and the interassay CV was < 9.4% at all concentrations.

Plasma concentrations of G-CSF were measured using a specific enzyme-linked immunoadsorption assay (ELISA) kit (Quantikine, RD systems, British Biotechnology, Cowley, Oxford, UK) and a 96-well plate reader (Dynatech MR700, Dynatech labs Ltd., Sussex, UK) as previously described [Watts et al. 1995]. The detection threshold of G-CSF was 5.0 pg/ml. Peripheral blood colony-forming cell assays for GM-CFC and burst-forming units-erythroid (BFU-E) were done using a commercially available methylcellulose-based medium (Methocult, Stem Cell Technologies Ltd., London, UK) as previously described [Watts et al. 1997]. Peripheral blood CD34-positive cells were counted by flow cytometry using a modified Siena protocol as previously described [Pollard et al. 1999].

Pharmacokinetic analyses

Maximum concentration (C_{max}) and time to maximum concentration (t_{max}) were observed values. Other pharmacokinetic parameters (half-life, $t_{1/2}$, AUC and clearance, Cl/F) were calculated by noncompartmental methods using WinNonlin software (version 3.0, Pharsight, Mountain View, CA, USA). Accumulation indices of C_{max} and AUC were calculated as ratio of values at presumed steady state (i.e. day 7) to the values on day 1 (see also below).

Statistical analyses

Data from all subjects who received CF101 were included in the analysis of safety

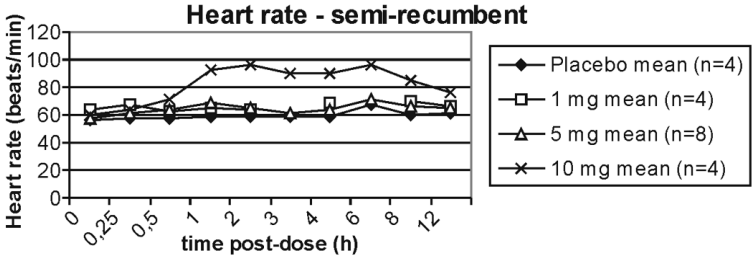

Figure 2. Time course and dose dependency of semirecumbent heart rate after single doses of CF101.

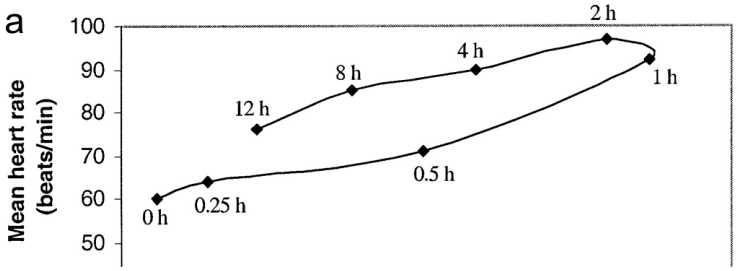

Figure 3a.

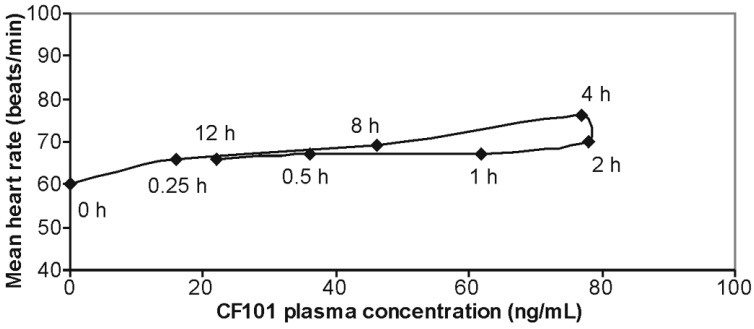

Figure 3b.

Figure 3. Modest development of hysteresis for concentration-dependent tachycardia after single oral doses of 10 mg CF101 (upper panel) but not 5 mg (lower panel); n = 4 unique subjects for each treatment group. Note the truncated vertical scales.

and tolerability (adverse events and laboratory safety variables). Numerical data and parameters were summarized using means or medians and other descriptive statistics, according to the type and distribution of the data. No hypothesis testing or formal dose-proportionality assessment was done, because of the small sample size at each dose level.

Results

Safety and tolerability

In the single-dose study, up to 5 mg oral CF101 was well-tolerated and without adverse findings in physical examination, vital signs, FEV_1, or ECG (12-lead and telemetry). In this dose range, there were also no clinically significant changes in clinical biochemistry, hematology, coagulation variables, or urinalysis. Apart from a single subject who reported a headache after 5 mg CF101, reported adverse events were restricted to the 10 mg treatment group, where headache was again reported, and the 2 subjects with the greatest increase in supine heart rate (see below) also experienced nausea and flushing; these adverse events were deemed treatment-attributable (see also Table 1). After placebo, there was no adverse event within 12 h.

In no subject was there a significant change in blood pressure, although this was not measured in the standing position. There was also no evidence of any change in ECG variables, including the PR interval, in any of the subjects. Sinus tachycardia was increasingly observable in the 2 highest dose groups. After

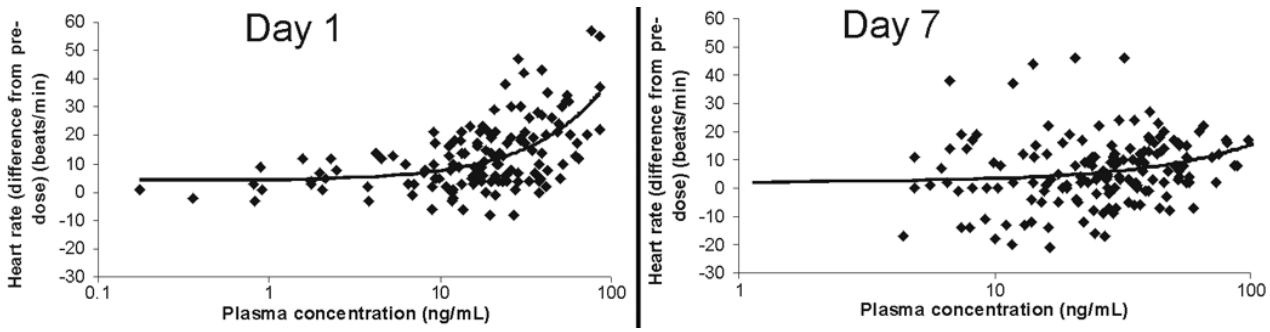

Figure 4. Attenuation of concentration-dependent tachycardia after multiple doses of oral CF101; n = 139 simultaneous plasma concentration and heart rate estimates (0 – 12 h) on day 1 (left hand panel), and n = 160 similar measures (0 – 24 h) on day 7 (right hand panel), in both cases from among n = 20 unique normal volunteers. Note the absence of correlation on day 7.

Table 1. Summary of adverse events reported after oral administration of CF101. Numbers of adverse events (numbers of subjects) are shown for each treatment group.

	Single dose			
	Placebo	1 mg solution	5 mg solution	10 mg solution
Headache			3 (3)	4 (4)
"Cold" symptoms	3 (1)		2 (1)	3 (1)
Flushing, lightheadedness on standing			1 (1)	1 (1)
Lower back pain	1 (1)		1 (1)	1 (1)
Nausea and vomiting				3 (2)
Red eyes			1 (1)	
Faint			1 (1)	
	(n = 4)	(n = 4)	(n = 8)	(n = 4)
Total number of events	4	0	9	12

	Multiple dose (7 days b.d.)				
	Placebo	2 mg suspension	3 mg suspension	4 mg suspension	5 mg suspension
Headache	2 (2)	2 (2)		1 (1)	4 (4)
Drowsiness	2 (2)				2 (2)
"Cold" symptoms	2 (2)		1 (1)		2 (2)
Discomfort at cannulation site	2 (2)				2 (2)
Flushing, dizziness on standing	1 (1)	–			2 (2)
Mouth ulcer	1 (1)	1 (1)			
Anxiety, tiredness	–				2 (2)
Facial rash with pruritus	1 (1)				
Musculoskeletal chest pain					1 (1)
Palpitations					1 (1)
	(n = 8)	(n = 5)	(n = 5)	(n = 5)	(n = 5)
Total number of events	11	3	1	1	16

5 mg CF101, the 4 active-treated subjects had increases in resting heart rate of 8 – 24 beats/min (bpm). After 10 mg, the 4 active-treated subjects had increases in resting heart rate of 18 – 55 bpm. The 2 subjects who experienced nausea and flushing (above) also had the largest increase in heart rate (pre dose 67 bpm to 115 bpm at 1 h post dose, and from pre-dose 50 bpm – 105 bpm 2 h post dose, respectively; the latter subject also vomited). These events precluded study of higher doses. The relationship between heart rate and plasma CF101 concentration is shown in Figures 1, 2, 3.

In the multiple-dose study, oral CF101 was well-tolerated and not associated with abnormalities in physical examination, vital signs, FEV_1 or ECG monitoring. There were no clinically significant changes in ECG variables or safety tests of blood and urine. There was a dose-related increase in heart rate on day 1, but some tolerance developed, because this effect was clearly smaller on day 7 (Figure 4). The day 1 time course of the increase in heart rate was consistent with a concentration-dependent effect, but the fact that heart rate changes were much smaller on day 7 made concentration-dependency analysis redundant.

Only 2 nonspecific adverse events were reported at any time during the 7 days of 12-hourly doses of up to 4 mg oral CF101. However, a dose-dependent increase in adverse event frequency was seen in the 5 mg treatment group. Among the 13 reported adverse events within 12 h after a dose, headache and

Table 2. Single-dose pharmacokinetics of oral CF101. Mean (SD) values are shown for peak concentration (C_{max}), area under the time-plasma concentration curve (AUC with time period subscript), half time of elimination ($t_{1/2}$), and plasma clearance (Cl/F). The time of C_{max} (t_{max}) is shown as median (range); n = 4 unique normal volunteers per dose group.

Dose (mg)	C_{max} (ng/ml)	t_{max} (h)	$AUC_{(0-48)}$ (ng.h/ml)	$AUC_{(inf)}$ (ng.h/ml)	$t_{1/2}$ (h)	Cl/F (l/h)
1	21.2 (2.1)	1 (1 – 2)	220.7 (20.9)	225.2 (21.7)	8.7 (0.7)	4.5 (0.4)
5	81.6 (23.6)	1 (1 – 2)	872.3 (211.6)	904.0 (221.9)	8.3 (0.2)	5.8 (1.4)
10	178.0 (46.6)	1 (1 – 2)	1780.0 (228.7)	1813.0 (226.5)	8.6 (0.4)	5.6 (0.7)

Table 3. Multiple-dose (12-hourly, days 1 and 7) pharmacokinetics of oral CF101. Abbreviations, mean and ranges as in Table 2; n = 5 unique subjects per treatment group, and different from those in Table 2.

Dose (mg)	Day	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-inf} (ng.h/ml)	$AUC_{(0-12)}$ (ng.h/ml)	$t_{1/2}$ (h)	Cl/F (l/h)
2	1	22.0 (3.3)	2 (1 – 4)	207.4 (52.0)	–	5.52 (0.2)	10.1 (2.3)
	7	30.9 (3.1)	2 (1 – 2)	–	242.4 (41.4)	9.83 (1.2)	4.9 (0.7)
3	1	49.3 (9.7)	2 (1 – 2)	423.5 (27.5)	–	6.29 (1.0)	7.1 (0.5)
	7	49.0 (7.9)	1 (1 – 2)	–	341.6 (38.1)	9.25 (0.8)	5.0 (0.6)
4	1	46.2 (11.4)	2 (1 – 2)	400.2 (85.7)	–	5.77 (0.6)	10.4 (2.1)
	7	58.1 (10.4)	1 (1 – 2)	–	458.3 (54.8)	8.93 (0.8)	5.4 (0.7)
5	1	63.6 (22.0)	2 (1 – 2)	596.1 (196.6)	–	4.96 (0.3)	9.3 (3.5)
	7	79.5 (24.1)	2 (2 – 2)	–	601.0 (163.6)	9.39 (0.6)	5.4 (1.5)

drowsiness were most common, and after the first dose there was a single subject with dizziness on standing and another with flushing. These compared with 7 adverse events with 12 h of a placebo dose, again most commonly headache (see Table 1).

Pharmacokinetics

The single-dose pharmacokinetics of oral CF101 are summarized in Table 2 and Figure 5. CF101 was absorbed promptly with t_{max} = 1 – 2 h in all cases. Mean C_{max} and AUC_{0-48} were linearly related to dose, and the half-life of elimination ($t_{1/2}$) was about 9 h and dose-independent. Apparent plasma clearance (Cl/F) was always 4 – 7 l/h and, again, dose-independent.

Table 3 and Figure 6 provide the multiple-dose pharmacokinetics of oral CF101. Again, CF101 was absorbed rapidly with t_{max} = 1 – 2 h. Trough plasma concentrations suggested that steady state was reached by day 3 at all dose sizes. Other pharmacokinetic parameters were similar after multiple-dosing to those observed after single doses: $t_{1/2}$ = 9 – 10 h and dose-independent, plasma concentrations of CF101 were dose-proportionate on days 1 and 7, and apparent plasma clearance remained dose-independent at 5 – 10 l/h (see Figure 7). Accumulation indices ranged between 1.0 – 1.4 and 1.1 – 1.6 for C_{max} and AUC, respectively, again consistent with the elimination kinetics observed after a single dose of oral CF101.

Pharmacodynamics

At 2 – 8 h after single doses of CF101, there was a dose-related increase in neutrophil count (Figure 8). Mean neutrophil count before dosing was 3.96×10^9/l (95% CI 3.74 – 4.18×10^9/l); mean neutrophil count at 4 h after 10 mg CF101 was 10.37×10^9/l (95% CI 7.55 – 13.19×10^9/l). Levels had returned to baseline values by 24 h after dosing. At 8 h after the first of the multiple doses, there was a similar dose-related increase in neutrophil count. Mean neutrophil count before dosing was 2.79×10^9/l (95% CI 1.94 – 3.64×10^9/l); mean neutrophil count at 8 h after 5 mg CF101 was 5.62×10^9/l (95% CI 4.38 – $6.87 \times$

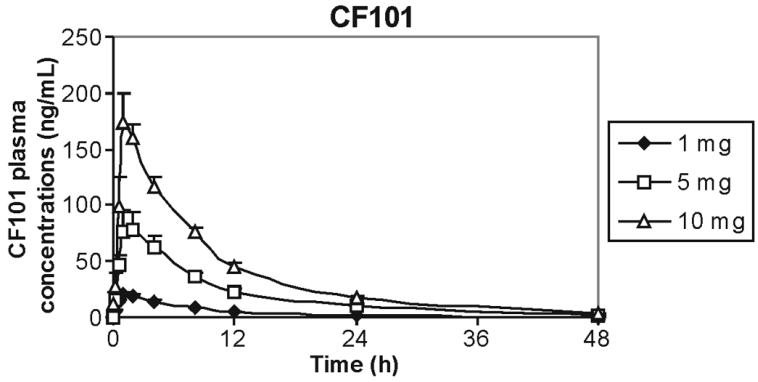

Figure 5. Mean (SEM) plasma concentration time profile after single oral doses of 1, 5 or 10 mg of CF101 to healthy men (n = 4 unique normal volunteers per treatment group).

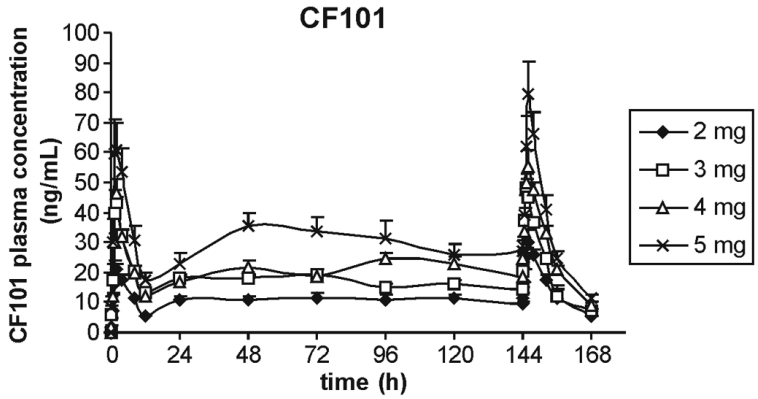

Figure 6. Pan-dose size stability of oral CF101 pharmacokinetics after 7 days of 12-hourly dosing. The first doses on day 1 (t = 0 h) and day 7 (t = 144 h) were accompanied by pharmacokinetic sampling at multiple, short intervals. During days 2 – 6, only trough plasma concentrations were measured. The day 1 and day 7 pharmacokinetics are indistinguishable, while trough levels do not increase after t = 48 h, consistent with the elimination kinetics of the drug (see Results). Mean (SEM) plasma concentrations shown, n = 5 unique normal volunteers per treatment group.

10^9/l). However, there was no clear dose relationship with dose on day 7 (Figure 9). There were no consistent changes in plasma G-CSF concentration, GM-CFC, or CD34 positive cell counts.

Discussion

These studies examined the safety, tolerability, pharmacokinetics and pharmacodynamic effects of oral CF101 in healthy men after single- and multiple (12-hourly × 7 days) doses. Single doses of CF101 up to 5 mg, and repeated doses of CF101 up to 4 mg 12-hourly, were safe and well-tolerated. Single doses of 10 mg CF101 were associated with flushing, tachycardia, nausea and vomiting; we treated these as dose-limiting, and avoided multiple 10 mg doses in the second study. Multiple 5 mg doses of oral CF101 were safe, but were associated with a greater adverse event frequency than were the excellently tolerated multiple doses of 4 mg. Adverse event types were similar in the 2 studies, with headache, drowsiness, hot flushes and dizziness on standing predominating. Most adverse events occurred around t_{max}. Although headache was prevalent among placebo-treated subjects, the time- and dose dependency of most of these adverse event types suggests drug attributability at the highest doses studied.

The pharmacokinetics of oral CF101 were linearly related to dose and did not change when multiple doses were introduced. Parenthetically, the 2 galenicals seem to have performed in a similar manner in these 2 studies, when we compared the single-dose data with those after the first of the multiple doses. This applies to both absorption and elimination kinetics when an expected steady state after day 3 in the multiple dose study was established. A mean dose-independent half-life of oral CF101 of about 9 – 10 h in both clinical trials suggests that 12-hourly dosing regimens are feasible in future studies.

Heart rate increases were concentration-dependent and dose-related, both after single doses and after the first of the repeated doses. In the absence of any important changes in semirecumbent or standing blood pressure after any dose of CF101, we presume that this is a direct effect, although the observation of flushing at the very highest doses does suggest vasodilation, at least in 1 vascular bed (the face)! The attenuation of this effect on day 7 of the multiple-dose study was in spite of the fact that there had been no diminution in CF101 plasma concentrations; this suggests both tolerance and reversibility of the effect. In any case, there were no ECG changes beyond a sinus tachycardia observed at any time in either study, and, in particular, the absence of any PR interval prolongation suggest that the compound lacks important A1 receptor effects at these concentrations.

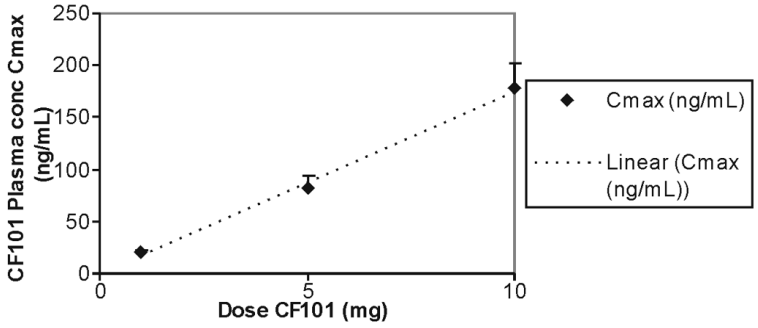

Figure 7a.

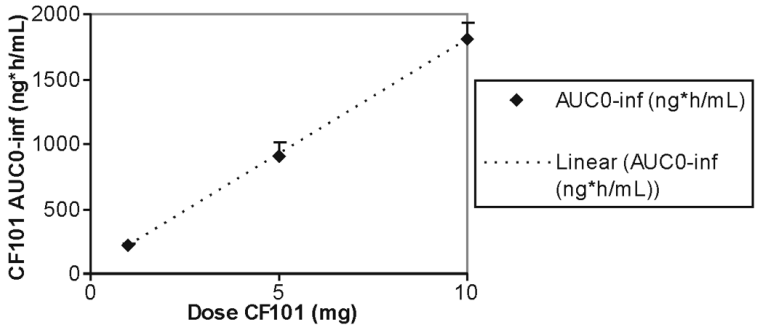

Figure 7b.

Figure 7. Dose proportionality of mean C_{max} (upper panel) and mean $AUC_{0\text{-}inf}$ (lower panel) after single oral doses of CF101 (mean ± SEM); n = 4 unique normal volunteers in each treatment group. R2 exceeds 0.98 in both cases, whether or not the origin is included in the correlation.

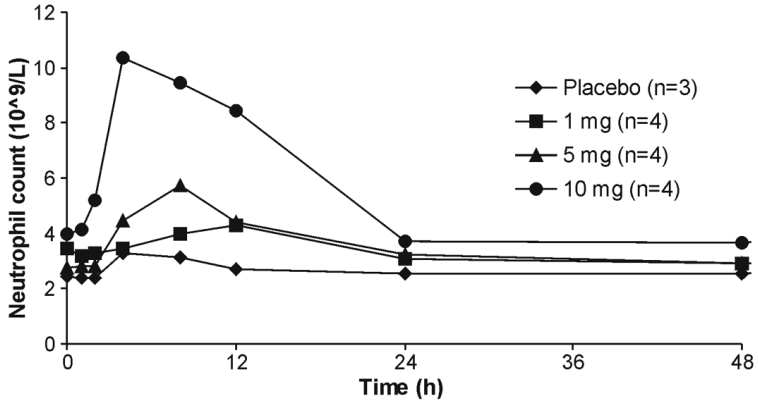

Figure 8. Dose dependency, time course and reversibility of neutrophil responses (10^9/l) after single doses of oral CF101.

Prior animal studies did not predict this reversible increase in heart rate. In reviewing the data, only in Cynomolgus monkeys is there a minor, but clinically insignificant, increase in heart rate 30 minutes after dosing. At the time, these were attributed to the stress of dosing, and we are inclined still to concur with that attribution because the doses used (10 and 100 mg/kg) were huge in comparison to those used in these clinical studies.

There are 2 prior observations that suggest that the observed tachycardia may not be entirely due to A3 receptor activation. Firstly, while Lasley et al. [1999] reported no change in ventricular function or heart rate in isolated rat or rabbit hearts, the isolated rat heart did respond to CF101 with an increase in coronary blood flow by 25%; this effect was partly attenuated by an A3 receptor antagonist and completely blocked by an A2a receptor antagonist, suggesting that, at high concentration CF101 might have some activity at the adenosine A2a receptor. Secondly, while there are no other published reports of the use of A3 or A2a agonists in healthy volunteers, Warrington et al. [personal communication] have noted dose-related increases in heart rate after oral dosing with an adenosine A2a receptor agonist. As in our studies, those increased heart rates were without significant changes in blood pressure, and were also sometimes accompanied by nausea, headache and lightheadedness. The highest plasma concentrations of CF101 in the present study did approach the in vitro Ki of the drug for the adenosine A2a receptor. Thus, it remains an open question whether high concentration, CF101-stimulated increases in heart rate in normal volunteers are mediated either by A2a or A3 receptor populations (or, conceivably, both). While the human being may possess adenosine receptor subtypes in the cardiovascular system that are species-dependent, the effect is highly predictable, seen at only relatively high dose and plasma concentration, is attenuated after multiple doses, and further study is needed to elucidate the pharmacological mechanism of action.

The transient leucocytosis after single doses of CF101 had a time course that clearly suggests drug attributability; its scale and reversibility, together with attenuation on day 7 in the multiple-dose study do not suggest any severe pathophysiological effect. The time course of the increase, together with an absence of effect on the cytokine concentrations or CD34-positive counts suggests that it was caused by release of neutrophils from the marginated pool into the circulating pool, rather than by bone marrow stimulation. This is unlike what is seen in mice, where increased neutrophil counts are accompanied

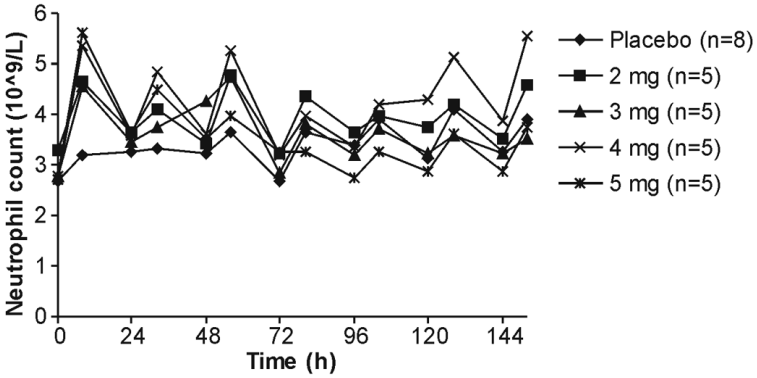

Figure 9. Absence of escalation and comparison to placebo-treated subjects of neutrophil response with 7 days of 12-hourly dosing of oral CF101.

by increased G-CSF secretion within 48 – 72 h after administering CF101 [Poulsen et al. 1998]. It is possible that a human cytokine and bone marrow response might take longer to develop, but this seems unlikely when not observed during 7 days of dosing.

Conclusion

Single oral doses of up to 5 mg and repeated oral doses of up to 4 mg CF101 given every 12 hours to healthy men were safe and well-tolerated. The pharmacokinetics of CF101 were linear, dose proportional and unchanged after repeated dosing. CF101 caused transitory increases in neutrophil count and heart rate linearly related to dose size. By day 7 of repeat dosing, the attenuation of cardiovascular effects suggests the development of tolerance. CF101 warrants further study for its efficacy in treating human disease.

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