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CG100649, a Novel Dual-Acting COX-2 and Carbonic Anhydrase Inhibitor: Ascending Single Dose and Multi-Dose Pharmacokinetics and Safety Evaluation in Healthy Male Subjects

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ABSTRACT

CG100649 is a novel dual-acting cyclooxygenase-2 (COX-2) and carbonic anhydrase (CA) inhibitor which is being developed for the treatment of osteoarthritis, rheumatoid arthritis, and acute pain. Single oral doses of 1 mg, 5 mg, 8 mg, and 12 mg of CG100649 were well tolerated when given to healthy male subjects (N=4 active and 2 placebo subjects per dose). There was statistically significant evidence for a linear dose-proportional increase, on average, in systemic exposure to CG100649 as measured by C_{max}. Single-dose CG100649 demonstrates a long terminal elimination half-life of approximately 5 days. Multiple doses consisting of a single loading dose followed by daily maintenance doses over 7 days (Group 1: 3 mg / 0.4 mg/day; Group 2: 6 mg / 0.8 mg/day; N=6 active and 2 placebo subjects per dose) demonstrated rapid achievement of steady state and consistent systemic exposure. After repeated daily dosing, the degree of accumulation of CG100649 in plasma (Ro) on Day 8 was not appreciably different from that observed on Day 1. On average, Ro values ranged from 1.1 to 1.2, consistent with rapid achievement of steady-state following a loading dose. Plasma CG100649 concentrations of 4 to 5 ng/mL (Group 1) and 6 to 9 ng/mL (Group 2) were maintained during the repeat-dose regimen. Maximum plasma concentrations of CG100649 were reached at approximately 3 to 12 hr post dose; thereafter, plasma CG100649 concentrations declined with a mean apparent terminal half life of approximately 100 to 109 h. Extrapolating from the preclinical efficacy data, the plasma concentrations with the higher maintenance dose regimen exceeded probable therapeutic concentrations by at least four fold. All doses were well tolerated. Use of a loading dose + reduced maintenance dose regimen may reduce the time to reach steady state plasma concentrations to approximately one day.

INTRODUCTION

CG100649 is a novel dual-acting cyclooxygenase-2 (COX-2) and carbonic anhydrase (CA) inhibitor which is being developed for the treatment of osteoarthritis, rheumatoid arthritis, and acute pain. The CA inhibitory activity of CG100649 is unlikely to affect CG100649's intended therapeutic effects since CG100649 is believed to dissociate from CA and increase its local concentration in tissues which have low CA activity, such as inflamed joints. Given that CAs are abundantly present in a variety of tissues and cells, the high affinity of CG100649 for CAs may significantly affect the tissue distribution profile. CG100649 is expected to show reduced COX-2 inhibition in tissues or cells highly enriched with CAs (GI tract, blood, and kidney) due to substantial uptake of CG100649 by CAs; this may provide protection for organs of toxicity concern for NSAIDs. These data represent the first-in-man Phase I data for CG100649 in healthy male volunteers. CG100649 is currently in phase II clinical trials in Europe.

METHODS

Two phase I studies were performed in the UK to investigate the safety, tolerability and pharmacokinetics (PK) of CG100649 administered orally to healthy male volunteers. The first study was a double-blind, placebo-controlled, randomized, single escalating dose study (1, 5, 8, or 12 mg) in four sequential groups of 6 healthy male subjects (24 total subjects; 2:1 active: placebo randomization). The second study was a double-blind, randomized, placebo-controlled study to evaluate the safety and PK of two oral multiple rising dose regimens of CG100649 administered as a loading dose (3 or 6 mg) followed by 7 daily doses (0.4 or 0.8 mg/day) which was designed to achieve rapid steady-state blood levels based on the initial Phase I data.

Table 1. Demography of single ascending dose study (Mean ± SD)

Parameter	Treatment (CG100649/placebo)				
	1 mg n=4	5 mg n=4	8 mg n=4	12 mg n=4	Placebo n=8
Age (yrs)	23.5 ± 3.1	28.3 ± 5.0	35.3 ± 8.0	26.5 ± 3.3	24.0 ± 5.9
Weight (kg)	72.2 ± 5.7	77.6 ± 1.7	78.0 ± 7.5	74.6 ± 10.5	83.9 ± 10
Height (cm)	177 ± 3.3	175 ± 2.9	180 ± 6.6	178 ± 4.8	184 ± 5.9
BMI (kg/m²)	23.0 ± 1.8	25.5 ± 1.3	24.0 ± 2.5	23.5 ± 2.1	24.6 ± 2.1

Table 2. Demography of multiple ascending dose study (Mean ± SD)

Parameter	Treatment (CG100649/placebo) #		
	3 mg / 0.4 mg/day n=6	6 mg / 0.8 mg day n=6	Placebo n=4
Age (yrs)	33.7 ± 4.2	31.7 ± 1.5	34.5 ± 3.4
Weight (kg	74.2 ± 6.2	73.1 ± 5.6	81.0 ± 2.0
Height (cm)	178 ± 4.4	178 ± 3.7	179 ± 2.5
BMI (kg/m²)	23.0 ± 2.1	23.0 ± 1.7	25.5 ± 0.6

Subjects received 3 mg on Day 1 and 0.4 mg on Days 2-8, or subjects received 6 mg on Day 1 and 0.8 mg on Days 2-8.

RESULTS

Singe Dose Study: CG100649 was well tolerated. A total of 14 treatment-emergent adverse events were reported by 8 of the 24 subjects; 25% of subjects in the treatment group and 50% of subjects in the placebo. There was only one gastrointestinal related adverse event (mild flatulence) following treatment with CG100649. There was no evidence of occult gastrointestinal blood loss. No clinically significant laboratory abnormalities were reported and there were no obvious trends in any of the laboratory data or in other safety including physical examination, vital signs, and 12-lead ECG.

Following single administration, plasma concentrations of CG100649 were generally measurable up to 144 hr (1 mg), 240 hr (5 mg), 384 hr (8 mg) and 480 hr (12 mg) post-dose. Max plasma concentrations were reached at approximately 5.5 to 72 hr post-dose (median estimates), then declined with a mean apparent terminal half-life of approximately 111 hr (5 mg) and 133 hr (12 mg).

There was statistically significant evidence for a dose-proportional increase, on average, in systemic exposure to CG100649 as measured by C_{max}.

The prolonged half-life would result in steady state plasma concentrations being reached over approximately 20 days of fixed dosing. However the good tolerance of higher doses enables an oral loading dose to be used to reduce the time to reach steady state plasma concentration in future clinical studies.

Table 3. Pharmacokinetic parameters of single ascending dose study Mean ± SD or Median (range)

Dose (mg)	C_{max} (ng/ml)	t_{max}+ (hr)	$AUC_{0-\infty}$ (ng.hr/ml)	$t_{1/2}$ (hr)
1 mg	1.57 ± 34.9	36.0 (6-120)	NC	NC
5 mg	7.79 ± 9.7	5.50 (3-48)	1500 ± 29 [3]	111 ± 53 [3]
8 mg	13.2 ± 11.1	8.00 (3-12)	NC	NC
12 mg	18.0 ± 12.3	72.0 (48-96)	4270 ± 18	133 ± 18

n=4 unless otherwise stated [ ]; NC = Not calculated.

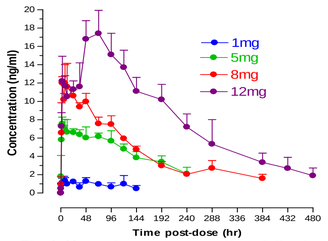

Fig. 1. Mean plasma concentrations following single oral administration of 1, 5, 8, or 12 mg CG100649

Multi-Dose Study: CG100649 was well tolerated. There were 24 treatment-emergent adverse events; 16 were reported by 8 subjects receiving CG100649 (67% of subjects) and 8 events were reported by 4 subjects receiving placebo treatment (100% of subjects). All adverse events were mild in intensity. No clinically significant laboratory abnormalities were reported and there were no apparent trends in the other safety parameters (physical examination, vital signs, 12-lead ECG.

Plasma concentrations of 4 to 5 ng/mL (Group 1) and 6 to 9 ng/mL (Group 2) were maintained during the repeat-dose regimen. Max plasma concentrations were reached at approximately 3-12 hr post-dose; thereafter, plasma CG100649 concentrations declined with a mean apparent terminal half-life of approximately 100-109 hr. Between-subject (inter-individual) variability in systemic exposure (AUC_{0-24}) was generally low, with coefficients of variation ranging from 12% to 30%. Following a loading dose + daily maintenance dose regimen, systemic exposure on Day 8 was not appreciably different from Day 1.

Table 4. Pharmacokinetic parameters of multiple ascending dose study. Mean ± SD or Median (range)

Group* (mg)	Day	C_{max} (ng/ml)	t_{max}+ (hr)	AUC_{0-24} (ng.hr/ml)	$t_{1/2}$ (hr)	R_o
1	1	4.61 ± 30.4	8.0 (3-24)	83.0 ± 30.3	NC	NC
	8	5.05 ± 18.1	5.5 (0.5-24)	96.4 ± 16.2	100 ± 23	1.2 ± 15
2	1	9.05 ± 20.6	12 (3-24)	148 ± 12.1	NC	NC
	8	8.43 ± 14.9	3.0 (0-48)	166 ± 16.3	109 ± 33	1.1 ± 24

n=6; NC = Not calculated.
* Group 1: Subjects received 3 mg on Day 1 and 0.4 mg on Days 2-8.
* Group 2: Subjects received 6 mg on Day 1 and 0.8 mg on Days 2-8.

Table 5. Daily pre-dose levels in the multiple ascending dose study. Mean ± SD

Dose	Day	Mean Pre-Dose Plasma Level (ng/ml)	
		3 mg loading + 0.4 mg/day for 7 days	6 mg loading + 0.8 mg/day for 7 days
Loading Dose	1	0	0
	2	3.77 ± 0.94	6.15 ± 0.87
	3	4.16 ± 0.99	6.19 ± 1.25
	4	3.98 ± 1.67	6.29 ± 1.21
Maintenance Dose	5	4.67 ± 1.13	6.50 ± 1.51
	6	4.52 ± 3.58	6.28 ± 0.85
	7	3.58 ± 0.58	6.56 ± 0.95
	8	3.64 ± 0.78	7.07 ± 2.26
	Daily Average	4.05	6.43

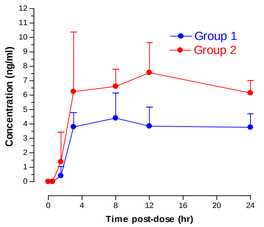

Fig. 2. Day 1 mean pre-dose plasma concentration-time profiles following 3 mg or 6 mg loading doses.

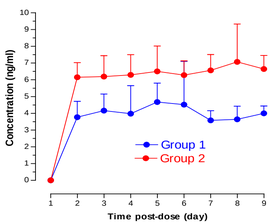

Fig. 3. Days 2-8 mean pre-dose plasma concentration-time profiles following 3 mg + 0.4 mg/day, or 6 mg + 0.8 mg/day.

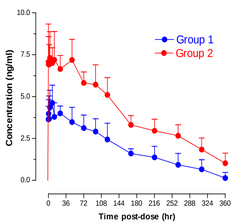

Fig. 4. Day 8-23 terminal plasma concentration-time profiles following final 0.4 or 0.8 mg dose on Day 8.

CONCLUSIONS

1. CG100649 was well tolerated by the healthy male subjects participating in this study.
2. The subject incidence of adverse events was higher in subjects receiving the lower dose (83% of subjects receiving 3 mg / 0.4 mg CG100649 compared to 50% of subjects receiving 6 mg / 0.8 mg CG100649). All adverse events were mild in intensity.
3. Plasma concentrations were measurable up to 480 hr after the 1st dose and up to 360 hr after the 8th dose.
4. Plasma CG100649 concentrations of 4 to 5 ng/mL (Group 1) and 6 to 9 ng/mL (Group 2) were maintained during the repeat-dose regimen. Maximum plasma concentrations of CG100649 were reached, on average, at approximately 3 to 12 hr post-dose.
5. The mean apparent terminal half-life was 100 to 109 hr.
6. Between-subject (inter-individual) variability in the extent of systemic exposure was generally low, with coefficients of variation ranging from 12% to 30%.
7. Systemic exposure to CG100649 on Day 8 was not appreciably different to that observed on Day 1.

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CG100649, a Novel Dual-Acting COX-2 and Carbonic Anhydrase Inhibitor: Multi-Dose Pharmacokinetics and Safety Evaluation in Healthy Male and Female Subjects

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ABSTRACT

Oral CG100649 was administered in 3 escalating loading and maintenance dose regimens for 5 days in 47 healthy male and female volunteers. Subjects in Cohort A received 2.0 mg (Day 1) followed by 0.3 mg/day (Days 2-5). Subjects in Cohort B received 4.0 mg (Day 1) followed by 0.6 mg/day. Subjects in Cohort C received 8.0 mg (Day 1) followed by 1.2 mg/day. Within each dose cohort, 12 subjects (6 male and 6 female) were randomized to receive active compound and 4 subjects (2 male and 2 female) received placebo. PK and safety evaluations continued through Day 31. All doses were well tolerated. CG100649 was well absorbed with linear dose-proportionality in among the 3 treatment groups. Inter-subject variability was low (generally 20% or less). Nearly all subjects achieved peak blood & plasma levels in 4-8 hr after the initial loading dose and maintained approximately steady-state blood levels for the remaining 4 days. Due to its preferential high-affinity binding to red blood cell carbonic anhydrase (CA), CG100649 showed 85-100x higher concentrations in whole blood than plasma in all 3 dose cohorts, in both males and females, from Day 1 through Day 5. The terminal half-life in both blood and plasma was 5-6 days. One female subject in Cohort C appeared to have faster drug elimination, so progressively declining blood levels were observed from Days 2-5; plasma levels were maintained at steady-state. No gender differences were apparent in drug exposure. Consistent with its potent CA inhibitory activity, dose-related increases were observed in serum chloride (females much more than males) and in plasma aldosterone (females possibly more than males). These data provide the first evidence of a COX-2 inhibitor with functional CA activity which may improve its overall safety profile by reducing effective free concentrations in tissues with high CA activity.

INTRODUCTION

CG100649 is a novel dual-acting cyclooxygenase-2 (COX-2) and carbonic anhydrase (CA) inhibitor which is being developed for the treatment of osteoarthritis, rheumatoid arthritis, and acute pain. Given that CAs are abundantly present in a variety of tissues and cells, the high affinity of CG100649 for CAs may significantly affect the tissue distribution profile. CG100649 is expected to show reduced COX-2 inhibition in tissues or cells highly enriched with CAs (GI tract, blood, and kidney) due to substantial uptake of CG100649 by CAs; this may provide protection for organs of toxicity concern for NSAIDs. CG100649 is believed to dissociate from CA and increase its local concentration in tissues which have low CA activity, such as inflamed joints. CG100649 is currently in phase II clinical trials in Europe.

METHODS

A Phase I double-blind, multi-dose, placebo-controlled study was conducted in 47 healthy male and female volunteers in the United States. The objectives were to assess the safety, tolerability, and pharmacokinetics of 3 escalating loading and maintenance dose regimens of CG100649 administered orally for 5 total days. Normal healthy male and female subjects (1:1 randomization within each treatment group) received a loading dose (Day 1) and 4 daily maintenance doses (Days 2-5) of 2 mg / 0.3 mg (Cohort A), 4 mg / 0.6 mg (Cohort B), 8 mg / 1.2 mg (Cohort C) or placebo. Active doses were designed to maintain quasi-steady-state blood levels during the dosing period. There were no Serious Adverse Events (SAEs) in any dose group.

Table 1. Demography of the study (Mean ± SD)

Parameter	Placebo N =12 (Female 6)	Active Treatment A N =12 (Female 6)	Active Treatment B N =11 (Female 5)	Active Treatment C N =12 (Female 6)	Total N =47 (Female 23)
Age (yrs)	26.2 ± 7.1	23.9 ± 8.8	26.7 ± 9.6	25.5 ± 10.3	25.6 ± 8.8
Weight (kg)	75.3 ± 15.4	72.6 ± 13.5	71.9 ± 12.3	79.3 ± 15.3	74.8 ± 14.0
Height (cm)	174 ± 7.6	176 ± 7.6	172 ± 12	176 ± 12	175 ± 9.8
BMI (kg/m^2)	24.8 ± 3.6	23.3 ± 3.0	24.2 ± 3.4	25.4 ± 3.7	24.4 ± 3.4

Table 2. Baseline subject characteristics at screening

		Placebo (N=12) N (%)	Active Treatment A (N=12) N (%)	Active Treatment B (N=11) N (%)	Active Treatment C (N=12) N (%)	Total (N=47) N (%)
Gender	female	6 (50.0%)	6 (50.0%)	5 (45.5%)	6 (50.0%)	23 (48.9%)
	male	6 (50.0%)	6 (50.0%)	6 (54.5%)	6 (50.0%)	24 (51.1%)
Ethnic origin	White	11 (91.7%)	12 (100.0%)	10 (90.9%)	11 (91.7%)	44 (93.6%)
	Black	1 (8.3%)	0 (0.0%)	1 (9.1%)	1 (8.3%)	3 (6.4%)
Smoking	smoker	1 (8.3%)	1 (8.3%)	2 (18.2)	0 (0.0%)	4 (8.5%)
	non-smoker	10 (83.3%)	10 (83.3%)	7 (63.6%)	12 (100.0%)	39 (83.0%)
	ex-smoker	1 (8.3%)	1 (8.3%)	2 (18.2)	0 (0.0%)	4 (8.5%)

CG100649 was well absorbed following oral administration. Nearly all subjects achieved peak blood and plasma concentrations in 4-8 hr after the initial loading dose and maintained approximately steady-state blood and plasma levels with daily maintenance dose for the remaining 4 days. Due to its preferential high-affinity binding to red blood cell carbonic anhydrase, CG100649 showed approximately 85-100 times higher concentrations in whole blood than plasma in all 3 dose cohorts, in both males and females, from Day 1 through Day 5. The mean terminal half-life in both blood and plasma ranged from 4-6 days.

Table 3. Blood PK parameters for CG100649 following single oral loading dose and 4 daily maintenance dose (N=6 per gender). Mean ± SD or Median (range)

Cohort Gender	Day 1 t_{max} [a] (hr)	Day 1 C_{max} (ng/ml)	Day 1 AUC_{0-24} (ng.hr/ml)	Day 5 t_{max} (hr)	Day 5 C_{max} (ng/ml)	Day 5 AUC_{0-24} (ng.hr/ml)	$t_{½}$ [b] (hr)	R_0 [c]
2 mg + 0.3 mg/day								
Male	7 (4-12)	275±23	5736±544	4 (2-6)	314±48	3833±968	154.4	1.19
Female	6	316±59	6277±1186	4 (2-24)	303±72	6638±1665	120.6	1.06
4 mg + 0.6 mg/day								
Male	8 (4-12)	519±98	10306±1900	3.5 (2-12)	551±89	11978±1945	147.9	1.17
Female [d]	8	573±45	11180±1056	4 (2-8)	538±49	11186±1287	103.4	1.01
8 mg + 1.2 mg/day								
Male	6 (3-8)	847±111	17816±2387	3.5 (0-4)	936±145	20260±2906	129.9	1.14
Female	6 (4-12)	1156±222	22402±3700	3.0 (0-4)	1091±232	21882±4452	116.2	0.98

a: median (range); b: $t_{1/2}$was estimated using data from Day 5 through Day 30 (if available);
c: R_0=AUC_{0-24} (Day 5)/AUC_{0-24} (Day 1), calculated using individual values; d: N=5

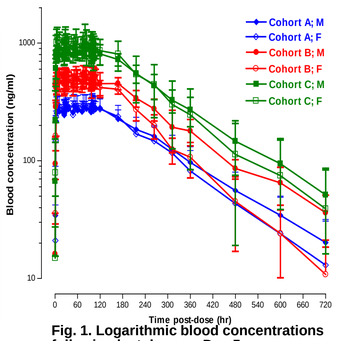

Fig. 1. Logarithmic blood concentrations following last dose on Day 5

Table 4. Plasma PK parameters for CG100649 following single oral loading dose and 4 daily maintenance dose (N=6 per gender). Mean ± SD or Median (range)

Cohort Gender	Day 1 t_{max} [a] (hr)	Day 1 C_{max} (ng/ml)	Day 1 AUC_{0-24} (ng.hr/ml)	Day 5 t_{max} (hr)	Day 5 C_{max} (ng/ml)	Day 5 AUC_{0-24} (ng.hr/ml)	$t_{½}$ [b] (hr)	R_0 [c]
2 mg + 0.3 mg/day								
Male	7 (4-12	2.9 ± 0.4	55 ± 6.4	7 (2-24)	3.6 ± 0.2	73 ± 4.6	140	1.3
Female	10 (6-24	3.7 ± 0.6	68 ± 11	8 (3-8)	4.1 ± 1.2	77 ± 16	137	1.1
4 mg + 0.6 mg/day								
Male	8 (4-8)	6.5 ± 0.7	121 ± 10	8 (0-24)	7.5 ± 1.5	147 ± 18	144	1.1
Female [d]	6(4-12)	7.8 ± 1.9	126 ± 15	4 (0.5-8)	8.0 ± 1.8	142 ± 19	101	1.1
8 mg + 1.2 mg/day								
Male	6 (4-8)	12 ± 2.2	213 ± 38	6 (2-8)	13 ± 2.6	265 ± 56	125	1.3
Female	8 (8-8)	18 ± 7.1	303 ± 99	6 (4-12	16 ± 3.3	330 ± 69	102	1.1

a: median (range); b: $t_{1/2}$was estimated using data from Day 5 through Day 30 (if available);
c: R_0=AUC_{0-24} (Day 5)/AUC_{0-24} (Day 1), calculated using individual values; d: N=5

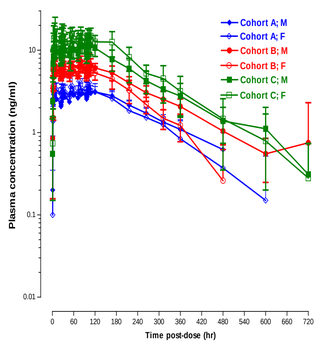

Fig. 2. Logarithmic plasma concentrations following last dose on Day 5

No clinically significant changes in laboratory parameters, physical examinations, vital sign parameters, physical examination results, or ECG recordings were reported in this study. The total number, the nature, and the severity of reported AEs were similar among the 4 treatment groups. All of adverse events were considered to be mild or moderate in severity and resolved by the end of the study. The percentage of adverse events that were considered related to the study drug was highest in the placebo group (3/12; 25.0%). The most frequently reported adverse events were headache (31% of active treatment subjects and 42% of placebo subjects), rash (23% of active treatment subjects and 33% of placebo subjects), ecchymosis (11% of active treatment subjects and 0% of placebo subjects), and pain in extremity (9% of active treatment subjects and 0% of placebo subjects).

No clinically significant ECG abnormalities occurred during the course of the study. One subject (0.6 mg/d) showed a “short burst of atrial fibrillation” on Day 5 of the Holter ECG monitoring, but follow-up evaluations indicated that the subject had no abnormal findings on stand alone ECG readings or continuous telemetry ECG readings. The safety review board determined that the Holter recording on Day 5 was an isolated incident. Systolic and diastolic pressures were very stable as depicted in Fig. 3.

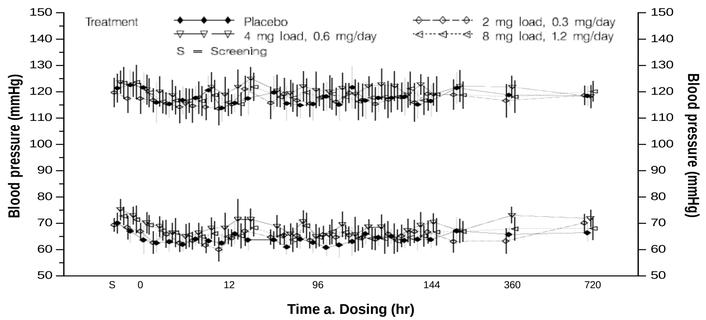

Fig. 3. Systolic and diastolic blood pressure (mmHg) – Mean course and SE bars by treatment, gender and time of all subjects

CONCLUSIONS

1. CG100649 was well tolerated in this U.S. Phase I study in 47 healthy male and female study.
2. All subjects achieved peak blood and plasma concentrations in 4-8 hr after the initial loading dose and maintained approximately steady-state blood and plasma levels with daily maintenance dose for the remaining 4 days.
3. CG100649 showed approximately 85-100 times higher concentrations in whole blood than plasma due to its preferential high-affinity binding to RBC carbonic anhydrase. No gender differences were apparent in drug exposure for cohorts A and B. In cohort C (high dose), exposure in female subjects was noticeably higher than in male subjects.
4. These data provide the first evidence for a COX-2 inhibitor with functional CA activity which may improve its overall safety profile by reducing effective free drug concentrations in tissues with high CA activity (blood vessel endothelial cells) while enhancing free drug concentrations in tissues with low CA activity (joints).

ACKNOWLEDGMENT

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CG100649, a novel dual-acting COX-2 and carbonic anhydrase inhibitor: Preclinical pharmacology

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ABSTRACT

CG100649 is a novel dual-acting cyclooxygenase-2 (COX-2) and carbonic anhydrase (CA) inhibitor which is being developed for the treatment of osteoarthritis, rheumatoid arthritis, and acute pain. CG100649 has moderate COX-2 selectivity over COX-1, ranging from 15-fold in human cells (whole blood, platelets and macrophages) to 45-fold in mouse peritoneal macrophages. In the ex vivo COX-1 activity assay, CG100649 showed weaker COX-1 inhibitory activity than indomethacin in rat whole blood. CG100649 was a potent inhibitor of inflammation in adjuvant-induced arthritis and collagen-induced arthritis in Lewis rats (paw swelling ED50s were 0.10 and 0.22 mg/kg/day, respectively). CG100649 and indomethacin showed similar potencies in the mouse acute air pouch and rat acute paw edema inflammatory animal models. CG100649 was 5x more potent than indomethacin in the thermal hyperalgesia rat model and had significantly greater anti-pyretic potency than ibuprofen. CG100649 inhibited hCA I and II activity with IC50s of 0.336 μM and 0.062 μM, respectively (acetazolamide IC50s were 0.68 μM and 0.0091 μM, respectively). The CA inhibitory activity of CG100649 is unlikely to affect CG100649's intended therapeutic effects since CG100649 is believed to dissociate from CA and increase its local concentration in tissues which have low CA activity, such as inflamed joints. Given that CAs are abundantly present in a variety of tissues and cells, the high affinity of CG100649 for CAs may significantly affect the tissue distribution profile. CG100649 is expected to show reduced COX-2 inhibition in tissues or cells highly enriched with CAs (GI tract, blood, and kidney) due to substantial uptake of CG100649 by CAs; this may provide protection for organs of toxicity concern for NSAIDs. It is possible that the high affinity of CG100649 for CA could produce a reduction in blood pressure similar to the potent CA inhibitor acetazolamide. CG100649 is currently in phase II clinical trials in Europe.

INTRODUCTION

Many NSAIDs are available for curing arthritic pain through the inhibition of COX-2 or COX-2. However, there is still a large unmet medical need for better antiinflammatory analgesics because adverse effects in the gastrointestinal (GI) track and cardiovascular (CV) system. Adverse effects on GI tract are believed to be due to inhibition of COX-1 that reduces the protective of prostaglandins on gastric and intestinal mucosa. On the other hand, adverse effects on the CV system may be caused by COX-2 inhibition that may result in thrombosis and vasoconstriction. We hypothesized that it may be possible to avoid GI and CV side effects by tissue-specific inhibition of COX-2. CG100649 is an orally available, small molecule dual inhibitor of COX-2 and carbonic anhydrase (CA). Oral administration of CG100649 produces significant levels of free active drug in joints, synovial fluid, and the CNS which are devoid of CA activity, but it is sequestered in an inactive form in many other tissues because the drug is tightly bound to CA-I and CA-II, a known family of proteins that are prevalent in the blood and in sites of potential COX-2 toxicity. CG100649 is currently under development for rheumatoid arthritis, osteoarthritis and acute pain.

IN VITRO PHARMACOLOGY

CG100649 is an orally available small molecule dual inhibitor of COX-2 and CA. The compound is a highly active inhibitor for COX-2 and is moderately selective for COX-2 over COX-1. It inhibits CAs at nM concentrations.

Table 1. Inhibitory activity of CG100649 against COX

Drug	Cells	IC50, ng/ml		COX-2 Selectivity
		COX-2	COX-1	
CG100649	PBMCs (COX-2)	0.97	14.9	15.3
	Platelets (COX-1)			
	Whole Blood	230	3,300	14.3
Indomethacin	PBMCs (COX-2)	1.8	6.1	3,3
	Platelets (COX-1)			
	Whole Blood	81	410	5.1
Rofecoxib	PBMCs	23		

- CG100649 is 23x more potent than rofecoxib vs. COX-2 in isolated PBMCs
- CG10649 shows a modest COX-2 selectivity over COX-1
- CG10649's effects are markedly attenuated in whole blood due to plasma protein binding and CA binding in RBCs

Table 2. Inhibitory activity of CG100649 against CAs

Drug	CA-I IC50, nM	CA-II IC50, nM	Remark [a,b]
CG100649	294	63	
Acetazolamide	630	16	
	250	12	Lit. value [c]
Celecoxib	50,000	21	Lit. value [c]

a. Assayed by the CA catalyzed hydrolysis of p-nitrophenylacetate in 5% DMSO aqueous buffer
b. Values of in-house study unless noted otherwise
c. Literature values cited from Weber A. *et al. J Med Chem* 2004, *47*, 550-557

* CG100649 is a potent inhibitor of CA-I & CA-II
* Celecoxib inhibits CA-II but it is almost inactive against CA-I
  - The assay medium contains 5% DMSO and does not reflect in vivo conditions

CG100649 is a dual inhibitor of COX-2 and carbonic anhydrase. The working hypothesis is that CG100649 cannot inhibit COX-2 in CA-rich tissues (CV & GI), but it fully inhibits COX-2 in CA-lacking tissues such as inflammatory joints. Thus, CG100649 should have reduced COX-2 effects in blood, blood vessels and kidneys by binding to CA and not binding to COX-2.

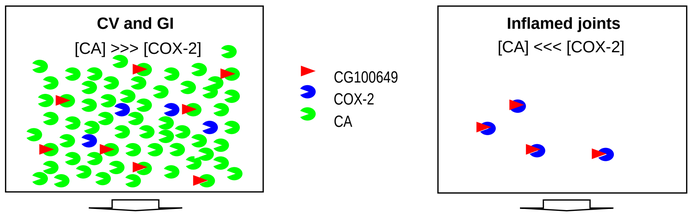


Figure 1. Working hypothesis for the tissue specific inhibition of COX-2 by dual inhibition of COX-2 and CA to avoid side effects of NSAIDs.

IN VIVO PHARMACOLOGY

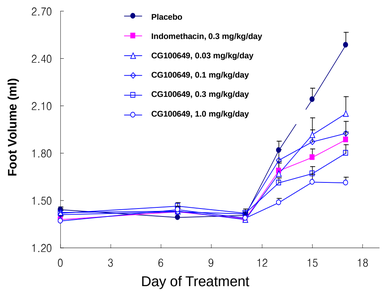
Table 4. Efficacy of CG100649 in the standard preclinical animal models.

Animal Model (male rats)	ED50a		
	CG100649	Celecoxib	Rofecoxib
Adjuvant-induced Arthritis [b]	0.1 mg/kg/day	0.5-1.0 mg/kg/day	0.74 mg/kg/day [c]
Collagen-induced Arthritis	0.2 mg/kg/day		
Hyperalgesia	0.25 mg/kg/day	35 mg/kg [c,e]	1.0 mg/kg [c,e]
LPS-induced Pyresis	59% @ 1 mg/kg	42% @ 30 mg/kg [c]	0.24 mg/kg [c]
Yeast-induced Pyresis	41% @ 1 mg/kg		
Rat Paw Edema [f]	> 3 mg/kg	7 mg/kg [c]	1.5 mg/kg [c]

a. Values of in-house studies unless noted otherwise; b. Preventive model & BID;
c. Drug manufacturer's data; d. Carrageenan-induced thermal hyper hyperalgesia;
e. by Randall-Selitto method; f. Carrageenan-induced rat paw edema.

CG100649 produces potent anti-inflammatory and analgesic activity in animal models of arthritis. It shows less activity against acute inflammation than chronic inflammation because of abundant presence of CAs in cells involved in acute inflammation. Thus CG100649 is suitable for use in chronic inflammation.

The OA therapeutic plasma level of CG100649 was estimated from the efficacy of CG100649 in the adjuvant-induced arthritis (AIA) model. In this model, the CG100649 showed superior efficacy to indomethacin and rofecoxib.



Drug	Dose [a]	Inhibition
CG100649	0.03	40 ± 10 %
	0.1	51 ± 9 %
	0.3	63 ± 9 %
	1.0	77 ± 4 %
Indomethacin	0.3	51 ± 4 %
Rofecoxib	ED_{50} = 0.74 mg/kg/day [b]	

a. 10 Lewis rats per group, mg/kg/day and BID
b. Merck data

Figure 2. Efficacy of CG100649 in the adjuvant-Induced arthritis (AIA) animal model.

CG100649 showed an ED_{50} of 0.1 mg/kg/day BID against AIA in male Lewis rats. Assuming linear pharmacokinetics for doses lower than 1mg/kg, the plasma C_{max} for 0.1 mg/kg/day BID may be extrapolated to be 4 ng/ml as Table 5.

Table 5. Plasma PK Data in Male SD Rats for CG100649 (po)

Dose	C_{max} ng/ml	$AUC_{0\text{-}24hr}$ ng•hr/ml
1.0 mg/kg	82	780
0.5 mg/kg	44	390
0.05 mg/kg	4	

Thus, the projected OA therapeutic plasma level of CG100649 is 4 ng/ml and the OA therapeutic plasma exposure is [4 ng/ml x 24 hr] or about 100 ng•hr/ml.

CONCLUSIONS

1) CG100649 is a highly active inhibitor of COX-2 which is moderately selective for COX-2 over COX-1. It also inhibits CAs in the nanomolar level. Such dual inhibition is designed to avoid COX-2 inhibition in CA-rich tissues (CV & GI) but fully inhibit COX-2 in CA-lacking tissues such as inflammatory joints.
2) CG100649 showed equal or higher efficacy and potency vs. celecoxib, indomethacin and rofecoxib in a variety of preclinical animal models such as adjuvant-induced arthritis (AIA) model.
3) The OA therapeutic plasma level of CG100649 is projected to be 4 ng/ml.

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CG100649, a novel dual-acting COX-2 and carbonic anhydrase inhibitor: Preclinical safety evaluations

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ABSTRACT

CG100649 is a novel dual-acting cyclooxygenase-2 (COX-2) and carbonic anhydrase (CA) inhibitor which is currently in phase II clinical trials in Europe. CG100649 was negative in the bacterial Ames assay and in the in vivo mouse micronucleus assay at oral doses up to 1250 mg/kg). The mutagenic potential of CG100649 was further investigated in the unscheduled DNA synthesis assay in rat liver. CG100649 administered to rats at oral doses of 62.5 and 125 mg/kg (male) and 7.5 and 15 mg/kg (female) failed to induce repairable DNA lesions in liver. Male rats tolerated target doses up to 5.0 mg/kg/day for 28 days, however females showed treatment-related histopathological findings in the intestines at target doses of 1.0 mg/kg/day and higher. The systemic exposure was greater in females rats which is commonly observed in rats due to a lower metabolizing capacity in females. The general activity and behavior in rats was not altered by the oral administration of CG100649 at single dose levels of up to 30 mg/kg. Similarly, oral treatment of CG100649 did not significantly affect the respiration rate or end tidal volume in conscious rats. Administration of oral doses up to 30 mg/kg CG100649 to awake cynomolgus monkeys had no marked effect on arterial blood pressure, heart rate or lead II ECG parameters (RR, PR, QT, QTcF and QTcQ intervals or QRS duration), waveform or rhythm in the 8 hours following dosing. Treatment with 2 μg/ml CG100649 in HEK293 cells stably transfected with hERG cDNA produced no inhibition of hERG tail current. In whole body radiography (QWBPI) studies in rats, the highest radioactivity was associated with whole blood and tissues with high blood perfusion such as liver, lung, kidney, and bone marrow which also have the highest CA activity. These data project a favorable safety profile for CG100649 in humans.

INTRODUCTION

Cyclooxygenase inhibition by classical non-steroidal anti-inflammatory drugs (NSAIDs) effectively treats acute and chronic pain. However, 2% to 4% of patients taking NSAIDs have symptomatic gastrointestinal (GI) ulcers and their complications. Further studies suggest that COX-2 inhibition mediates the anti-inflammatory effects of NSAIDs, whereas COX-1 inhibition is responsible for adverse effects on the GI tract. This lead to the development of COX-2 selective inhibitors (coxibs) that have the same anti-inflammatory benefits of nonselective NSAIDs but fewer GI side effects.

Effects of COX-2 inhibition on the cardiovascular (CV) system are not straightforward. Inhibition of COX-2–derived PGI_2 removes a protective constraint on thrombogenesis, hypertension, and atherogenesis *in vivo* (2) leading to an elevated risk of myocardial infarction and stroke. Thus, all commercially available NSAIDs and coxibs produce adverse GI or CV side effects in susceptible patients. Since the CV side effects of NSAIDs may be generated by COX-2 inhibition, we hypothesized that tissue-specific inhibition of COX-2 in inflamed joints may be a novel way to prevent adverse CV side effects in vascular tissues.

CG100649 achieves tissue specific inhibition of COX-2 through dual inhibition of COX-2 and carbonic anhydrase (CA). As summarized in the Figure 1, the working hypothesis is that CG100649 cannot inhibit COX-2 in CA-rich tissues (CV & GI), but it can fully inhibit COX-2 in tissues that lack CA activity such as inflammatory joints. CG100649 binds preferentially to CA in blood, blood vessels, and kidneys. In inflamed tissues, CG100649 is a highly active COX-2 inhibitor which is moderately selective for COX-2 over COX-1. In preclinical analgesia and antiinflammatory models, CG100649 is more potent than indomethacin, celecoxib and rofecoxib. The projected clinical therapeutic plasma level of CG100649 is estimated as 4 ng/ml on the basis of the efficacy in the adjuvant-induced arthritis (AIA) model in rats.

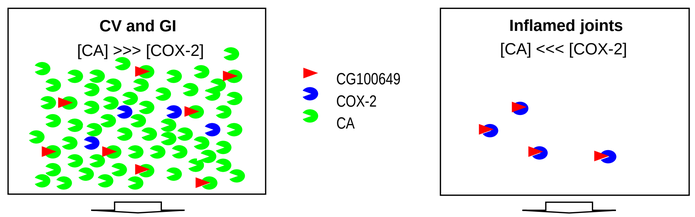

Figure 1. Working hypothesis for the tissue specific inhibition of COX-2 by dual inhibition of COX-2 and CA to avoid side effects of NSAIDs.

RESULTS

CG100649 has completed the following the GLP toxicity studies in the UK or US:

Table 1. GLP Toxicity studies of CG100649

Animal Toxicity Studies	Status
Safety pharmacology studies	completed
Genetic toxicity studies	completed
Single dose toxicity studies	completed
Repeat dose toxicity studies **4-week rat** **4-week monkey** **90-day rat** **90-day monkey**	completed
Developmental toxicity studies	completed
Reproductive toxicity studies	completed
Dog renal studies	completed

In vitro genotoxicity studies showed that CG100649 does not induce reverse mutations in the Ames test. However, it increased chromosomal aberrations in CHO cells at 200 μg/mL which also produced 50% cellular toxicity. *In vivo* studies showed that CG100649 did not produce genotoxic effects in the mouse bone marrow micronucleus test or in the unscheduled DNA synthesis (UDS) assay in rat liver. The weight of evidence suggests that the genotoxicity risk is low.

Oral 4-week toxicokinetic studies in rats at doses up to 3-5 mg/kg/day showed that systemic exposure in females was greater than in males due to the longer half-life in females (9-12 hr) compared to males (4-7 hr). A sex related difference in systemic exposure is commonly observed in rats and is usually attributed to a lower metabolizing capacity of female rats. In male and female monkeys, following single and repeat oral doses of 12 mg/kg/day, C_{max} was generally reached in 2- 4 hours post dose and the terminal half-life was shown to approximately 60 hr. Systemic exposure at week 4 was greater than on Day 1 (the degree of accumulation, R_o, was approximately 1.6 to 3.8). The overall extent of exposure was not appreciably different in male and female monkeys.

The oral maximum tolerated dose (MTD) for CG100649 was determined to be approximately 1500 mg/kg in mice. In rats, the MTD was 125 mg/kg in males and 15 mg/kg in females. Clinical findings were generally of GI nature.

Oral safety studies were conducted in rats and monkeys for 28 days. In male rats, target doses up to 5.0 (3.6 actual dose) mg/kg/day were well tolerated and produced no obvious signs of toxicity. Female rats showed treatment-related histopathological findings in the intestines at target doses of 1.0, 1.5 and 3.0 mg/kg/day. Cynomolgus monkeys treated at 12 mg/kg/day showed a low incidence pathological finding in the stomach and in blood chemistry. At 5 mg/kg/day, blood chemistry changes were noted in one male only. On the basis of these findings it was concluded that 2 mg/kg/day was a no observed effect level (NOEL), and 5 mg/kg/day was a no observed adverse effect level (NOAEL).

Table 2. Results of 4 Week repeat dose toxicity study of CG100649 in monkey

Sex	Daily Dose	Plasma Exposure (Day 28)	NOAEL
Male	2, 5 and 12 mg/kg/day	(2,420 ± 1,260) – (12,500 ± 9,770) ng•hr/ml	5.0 mg/kg/day (MTD ≥ 12 mg/kg/day)
Female		(2,010 ± 1,180) – (12,700 ± 8,610) ng•hr/ml	

Wide GI safety margins were observed despite the modest COX-2 selectivity. Rats did not show gastric findings even at an exposure of 30,000 ng•hr/ml. Monkeys did not show gastric findings up to an exposure larger than 20,000 ng•hr/ml.

Safety margins were estimated for OA treatment, assuming that the therapeutic plasma level is 4 ng/ml and daily therapeutic exposure is 100 ng•hr/ml.

Table 3. GI safety margins of CG100649

Safety Margin	Rat	Monkey	Remark
Intestinal	44	29	Estimated from NOAEL exposure
Gastric	> 300	≤ 200	Calculated from exposure with gastric finding

CONCLUSIONS

1) Safety pharmacology, genetic toxicity, single and repeat dose toxicity (4-week and 90-day studies in rat and monkey), developmental toxicity, reproductive toxicity, and dog renal studies show a favorable safety profile for CG100649.

2) CG100649 produced large GI safety margins in rats and monkeys despite its modest COX-2 selectivity over COX-1. The tight binding of CG100649 to carbonic anhydrase (CA) in the GI tract may be responsible for its good GI safety.

3) Small effects on the renal hemodynamics suggest negligible COX-2 inhibition in the renal vasculature owing to the significant enrichment of CAs in the renal vasculature. Likewise, COX-2 inhibition is expected to be low in other types of blood vessels conferring a benefit for CV safety

REFERENCES

1) Silverstein FE, Faich G, Goldstein JL, et al. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: a randomized controlled trial. Celecoxib Long-term Arthritis Safety Study. *JAMA.* 2000;284:1247–55.

2) Grosser T, Fries S, FitzGerald GA. Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. *J. Clin. Invest.* 2000;116:4–15 .

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