Analysis of safety and pharmacokinetic data derived from a single ascending dose Phase 1 study involving a promising non-cytotoxic anti-cancer agent Sulforadex®

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Introduction

It is widely documented in the literature that consumption of cruciferous vegetables (family *Cruciferae*) containing specific compounds are capable of suppressing the initial phase of carcinogenesis or the progression of neoplastic cells to cancer[1]. Sulforaphane, a derivative of glucoraphanin, was found to be one of such compounds. Several epidemiological studies have demonstrated that intake of cruciferous vegetables containing sulforaphane might reduce the risk of prostate cancer[2]. It has been established in literature that sulforaphane inhibits histone deacetylase, which is up-regulated in certain types of cancer including prostate cancer[3]. In human patients, it has been documented that decreases in histone acetylation is associated with increased cancer malignancy and risk of prostate cancer reoccurrence[4].

Pharmacokinetic (PK) studies have revealed that sulforaphane can reach detectable plasma concentrations in the blood. In rats, following a 50 µmol gavage of sulforaphane, sulforaphane levels peaked at approximately 20 µM after 4 hours with a $t_{1/2}$ of 2.2 hours[5]. In human subjects given single doses of 200 µmol broccoli sprouts isothiocyanate preparation, the maximum plasma concentration (C_{max}) peaked between 0.9 – 2.3 µM (1 hour after feeding) with a terminal elimination half-life ($t_{1/2}$) of 1.77 ± 0.13 hours[6]. Sulforadex® is a proprietary product composed of synthetic sulforaphane and α-cyclodextrin. While the initial indication is the prevention of progression of early prostate cancer, other indications may follow. Evgen is currently developing Sulforadex® as a non-cytotoxic product with low toxicity and few side effects anticipated in healthy individuals or cancer patients.

Although there are many clinical studies in progress with sulforaphane derived from botanical sources[7,8], to date there is no clinical experience with Sulforadex®. In this double blind, placebo controlled single ascending dose Phase 1 study, 29 healthy male subjects received single oral doses (50 mg, 100 mg, 300 mg, 500 mg or 700 mg) of Sulforadex® or placebo to assess its safety and PK in healthy male volunteers.

Aims

The aim of this study was to evaluate the safety and PK of single ascending doses (50 mg, 100 mg, 300 mg, 500 mg or 700 mg) of Sulforadex® in healthy male volunteers.

Methods

This was a Phase 1, randomised, double-blind, placebo-controlled study with single ascending doses of Sulforadex® administered to healthy male subjects between 18 to 45 years of age. There were a total of five Cohorts in the study. Five (5) healthy subjects (three on active [A]; two on placebo [P]) participated in Cohort 1 (50 mg dose level), four healthy subjects (three on A; one on P) participated in Cohort 2 and 3 (100 mg and 300 mg dose level) and eight healthy subjects (six on A; two on P) participated in Cohort 4 and 5 (500 mg and 700 mg dose level), and received Sulforadex® or placebo.

Each healthy male subject received verbal (from a Research Physician) and written information prior to signing of the Informed Consent Form. Subjects were screened within 21 days prior to first dosing and there was one in-house period to the Clinical Pharmacology Unit (CPU). Subjects were admitted to the CPU on study Day -1 and remained in-house until discharge on Day 3 with a follow-up visit performed 7-14 days after discharge. Administration of the study drug was on Day 1 with safety monitoring and serial blood samples for PK evaluation.

Statistical Methods

Adverse events (AEs) were tabulated according to Medical Dictionary for Regulatory Activities (version 15.1), primary system organ class and preferred term. The results of AE recording, vital signs, 12-lead ECG and standard clinical laboratory safety tests were listed by subject and analysed by descriptive statistics as appropriate.

PK data was listed for each subject, along with univariate statistics including arithmetic and geometric means, standard deviations (SD), minimum, maximum and median values, and inter-subject coefficients of variation (CV). Dose proportionality was analysed with a linear regression model using the logarithm of a PK parameter as the responsible variable and the logarithm of the dose as the independent variable.

Results

The descriptive statistics for demography parameters for all enrolled subjects is presented in Panel A (Table 1).

A Table 1: Summary of Demographic Data

Variable	Summary Statistics	Sulforadex® 50 mg	100 mg	300 mg	500 mg	700 mg	Placebo	Overall
n		3	3	3	6	6	8	29
Age (years)	Mean±SD	29.3±10.11	34.0±8.54	21.3±2.51	27.8±6.14	27.0±6.69	29.3±4.56	28.2±6.51
Body height (cm)	Mean±SD	179.3±5.13	180.6±3.78	179.3±2.88	176.8±5.84	180.3±8.38	179.2±4.97	179.1±5.48
Weight (kg)	Mean±SD	71.1±14.27	66.7±3.20	72.6±1.61	69.4±2.93	74.0±2.42	75.5±5.76	72.3±5.94
BMI (kg/m²)	Mean±SD	22.0±3.20	20.4±0.45	22.6±0.85	22.2±1.22	22.8±1.72	23.5±1.35	22.5±1.69
Race	Hispanic	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (12.5%)	1 (3.4%)
	Non-hispanic	3 (100%)	3 (100%)	3 (100%)	6 (100%)	6 (100%)	7 (87.5%)	28 (96.6%)

SD (Standard Deviation)

PK Analysis

Plasma Concentration-Time Profiles

One of the objectives of this study was to study the plasma PK of sulforaphane from Sulforadex® in healthy male volunteers. The median t_{max} of sulforaphane was found to occur between 0.63 - 1.00 hours, and the sulforaphane mean $t_{1/2}$ values ranged between 1.21 hours and 2.15 hours. The distribution and elimination phases appeared to be similar. For some dose levels (50 mg, 100 mg, 300 mg and 500 mg) the full complement of results for every parameter could not be achieved because plasma concentrations of sulforaphane were below the lower limit of quantification (LLOQ) which meant that there was insufficient data for the estimation of some PK parameters.

Pharmacokinetic Parameters

The summary statistics for the plasma PK parameters, C_{max}, t_{max}, AUC_{0-t}, $AUC_{0-\infty}$, K_{el} and $t_{1/2}$ are presented in Panel B (Table 2) for sulforaphane.

B Table 2: Summary of plasma PK parameters for sulforaphane following oral administration of 50 mg, 100 mg, 300 mg, 500 mg and 700 mg Sulforadex® in healthy male subjects

Treatment	n[#]	C_{max}[a] (ng/mL)	t_{max}[b] (hr)	AUC_{0-t}[a] (ng hr/mL)	$AUC_{0-\infty}$[a] (ng hr/mL)	K_{el}[a] (1/hr)	$t_{1/2}$[a] (hr)
50 mg	3	8.53 -28.06	0.79 (0.50, 1.00)	8.41 -60.8	-[c]	-	-
100 mg	3	35.69 -17.68	1.01 (1.00, 1.02)	58.34 -12.33	-	-	-
300 mg	3	22.26 -29.46	0.63 (0.50, 1.00)	55.26 -25.15	-	0.32 n=1[¥]	2.15 n=1[¥]
500 mg	5	37.95 -19.84	1 (0.50, 2.00)	74.58 -38.44	114.1 n=1[¥]	0.57 n=1[¥]	1.21 n=1[¥]
700 mg	6	105.53 -14.64	1 (0.50, 1.50)	247.73 -35.76	272.28 -34.87 n=5[π]	0.53 -21.3 n=5[π]	1.3 -21.3 n=5[π]

[a] Geometric Mean (Geometric CV [%]).
[b] Median (Min, Max).
[c] In some instances the plasma PK parameters could not be calculated because the plasma concentrations were below the LLOQ.
[#] Number of values used to calculate the mean for the given PK parameter.
For some parameters in the 500 and 700 mg treatment Cohort the number of values used to calculate the mean was n=1[¥] or n=5[π].

Dose Proportionality Analysis

Dose proportionality was analysed with a linear regression model using the logarithm of the PK parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ as the responsible variables and the logarithm of the dose as the independent variable. Based on the linear regression model, the dose proportionality coefficient (slope) and its two-sided 90% confidence interval (CI) was estimated. Dose proportionality would have been declared if 90% CI for the slope was completely contained in the range (1+log[0.5]/log[r], 1+log[2]/log[r]); where r = high dose/low dose. The dose proportionality analysis revealed that the 90% CIs for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ fell outside of this range and therefore the dose proportionality could not be established for these PK parameters (Panel C, Table 3).

C Table 3: Dose proportionality analysis results for Sulforadex®

Parameter	Slope*	90% Confidence Interval Lower	90% Confidence Interval Upper	Range# Lower	Range# Upper
C_{max} (ng/mL)	0.713	0.504	0.921	0.915	1.084
AUC_{0-t} (ng.hr/mL)	0.988	0.741	1.236	0.915	1.084
$AUC_{0-\infty}$ (ng·hr/mL)	2.584	0.233	4.935	0.915	1.084

*Slope is the dose proportionality coefficient
#Range (1+log[0.5]/log[r], 1+log[2]/log[r]); where r = high dose/low dose

Safety Evaluation

Adverse Events

There were no serious adverse events (SAEs) reported during the study and no subject was withdrawn from the study due to safety reasons. An overview of AEs and drug related AEs by treatment group are presented in Panel D (Table 4). One treatment emergent AE was reported in 1 of 3 (33.3%) subjects for 50 mg Sulforadex®, three AEs reported in 3 of 6 (50.0%) subjects for 500 mg Sulforadex® and four AEs reported in 4 of 6 (66.7%) subjects for 700 mg Sulforadex®. All treatment emergent AEs were drug-related AEs. No treatment emergent AEs were reported for the 100 mg and 300 mg Sulforadex® treatment groups. For the placebo group, two treatment emergent AEs were reported in 2 of 6 (25.0%) subjects and 1 subject (12.5%) was reported as having one treatment related AE.

D Table 4: Summary of Adverse Events

	Sulforadex® 50 mg (n=3)	100 mg (n=3)	300 mg (n=3)	500 mg (n=6)	700 mg (n=6)	Placebo (n=8)	Overall (n=29)
Number of subjects dosed	3	3	3	6	6	8	29
Number of subjects with at least one AE	1 (33.3%)	0 (0.0%)	0 (0.0%)	3 (50.0%)	4 (66.7%)	2 (25.0%)	10 (34.5%)
Number of subjects with at least one serious AE	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Number of subjects with at least one drug-related AE	1 (33.3%)	0 (0.0%)	0 (0.0%)	3 (50.0%)	4 (66.7%)	1 (12.5%)	9 (31.0%)
Number of subjects with at least one serious drug-related AE	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Number of AEs	1	0	0	6	8	2	17

Percentages in parentheses within tables are calculated by dividing the number of subjects with at least one AE by the number of subjects in the treatment group.

Clinical Laboratory Safety Parameters

Overall, there were no significant changes in the biochemistry, haematology, coagulation and urinalysis parameters. Some subjects were found to have values that were slightly elevated but none were considered to be clinically significant.

Vital Signs and 12-Lead ECGs

Some subjects were noted to have had values outside the normal range in vital signs and 12-lead ECGs. However, the isolated departures from the normal ranges in vital signs and 12-lead ECG recordings were considered by the Investigator as not clinically significant and not related to the studied drug.

Discussion

The safety findings from the study demonstrated that there were no SAEs and no subject withdrawals due to AEs or any other reasons. In addition, the findings showed that oral doses up to 300 mg Sulforadex® were safe and well tolerated. However, oral doses of 500 mg and 700 mg Sulforadex® were not as tolerated, as evidenced by the review of AEs. The majority of reported AEs were of mild intensity and four (vomiting and abdominal pain) were of moderate intensity. Notably, the study has shown that oral doses of 500 mg and 700 mg Sulforadex® were associated with an increasing frequency of vomiting in subjects.

The PK data demonstrated that the mean plasma concentrations of sulforaphane following administration of single doses of Sulforadex® (50 mg, 100 mg, 300 mg, 500 mg and 700 mg) in subjects appeared to be dose linear. C_{max} and AUC_{0-t} appeared to be dose linear; however the variability in the PK data particularly at the 100 mg and 300 mg dose levels precludes definitive conclusions regarding dose linearity from being made. In addition, dose proportionality was not established for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$.

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