A phase 1 dose-escalation study: safety, tolerability, and pharmacokinetics of FBS0701, a novel oral iron chelator for the treatment of transfusional iron overload

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The online version of this article has a Supplementary Appendix.

ABSTRACT

Background

There is still a clinical need for a well-tolerated and safe iron chelator for the treatment of transfusional iron overload. We describe the pharmacokinetic properties and safety data after 7 days of dosing of FBS0701, a novel oral, once-daily iron chelator.

Design and Methods

This phase 1b dose-escalation study to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of FBS0701, a novel oral iron chelator for the treatment of transfusional iron overload, was conducted in 16 adult patients with iron overloaded consequent to transfusions. FBS0701 was given daily for 7 days at doses up to 32 mg/kg and was well tolerated at all dose levels.

Results

Pharmacokinetics showed dose-proportionality. The maxium plasma concentration (C_{max}) was reached within 60-90 minutes of dosing and the drug was rapidly distributed at the predicted therapeutic doses. The plasma elimination half-life ($t_{1/2}$) was approximately 19 hours. There were no serious adverse events associated with the drug.

Conclusions

On the basis of these safety and pharmacokinetic data, FBS0701 warrants further clinical evaluation in patients with transfusional iron overload. *(Clinicaltrials.gov identifier: NCT01186419)*

Key words: FBS0701, phase I study, dose-escalation, oral iron chelator.

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Introduction

Morbidity from iron overload remains a major problem in transfusion-dependent patients despite recent advances in chelation therapy. Iron chelators in current use – parenteral deferoxamine, oral deferasirox and deferiprone – are individually efficacious in many patients with transfusion-dependent anemias, but each has limitations relating to safety, ease of administration, patient acceptance, or a narrow therapeutic index.[1] The novel oral chelator FBS0701, a member of the desazadesferrithiocin class of siderophore-related tridentate chelators, binds Fe(III) with very high affinity and selectivity over Fe(II) and other biologically important metals.[2-4] FBS0701 was selected for clinical development on the basis of its primary pharmacological and pharmacokinetic properties and the toxicity profile, all of which suggested that FBS0701 might offer significant clinical advantages for patients with iron overload as compared to currently available therapies.[5] Extensive preclinical toxicological studies of FBS0701 consistently demonstrated a higher no-observable-adverse-effect level (NOAEL) compared to deferasirox (ExJade®, Novartis) suggesting a favorable clinical safety profile especially with respect to nephrotoxicity.[6,7] In this study, we report the results of the first multi-dose exposure of FBS0701 in iron-overloaded patients.

Design and Methods

This study was conducted in accordance with the World Medical Association Declaration of Helsinki, Good Clinical Practice (GCP), and International Clinical Harmonization (ICH) guidelines and all applicable regulations governing human subject protection and was approved by Institutional Review Boards or local ethics committees at each site.

The study was conducted to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of FBS0701 in patients with transfusional iron overload. This was a multi-center, open-label study of ascending multiple oral doses of FBS0701. Four cohorts of four patients each were dosed sequentially with FBS0701. Doses of 3 mg/kg, 8 mg/kg, 16 mg/kg, or 32 mg/kg were given once daily for 7 days. The 7-day duration of the study was considered sufficient to accurately assess the pharmacokinetics of FBS0701 at steady state plasma levels.

Patients

Patients 18 years or older with documented transfusional iron overload requiring chronic treatment with deferiprone, deferasirox or deferoxamine were eligible. For the purposes of this study, transfusion dependence was defined as requiring eight or more transfusions per year. The iron burden inclusion criteria were defined as: (i) liver iron concentration greater than 2 mg/g (dry weight, liver) as determined by R2 magnetic resonance imaging; (ii) a cardiac magnetic resonance imaging T2* greater than 12 ms; and (iii) serum ferritin in excess of 600 ng/mL.

Eligible patients who consented to participation in the study discontinued their regular chelation therapy for up to 5 days during a wash-out period prior to their first dose of FBS0701. Dose cohorts and diagnoses are shown in Table 1. Patients received FBS0701 in capsules taken orally in a fasted state. In this study, a standard pharmacokinetic protocol of plasma sampling was performed throughout the 7 days of dosing and continuing for 3 days after the last dose on day 7.

Each dose level was separated by a minimum of 3 weeks to assess safety and tolerability prior to escalating to the next dose level. Following initial dosing on day 1, patients were followed in the clinic on days 3, 6, 7, 8, 9, 10 and 15 and then weekly thereafter for 3 weeks for a total of 28 days following the last dose.

Table 1. Demographics and hematologic diagnoses of iron-overloaded patients.

Cohort 1 (3 mg/kg)	2 β-thalassemia major 2 sickle cell disease	Mean age 19.3	M/F 1/3
Cohort 2 (8 mg/kg)	1 α–thalassemia ($\alpha^0\alpha^0$) 2 β-thalassemia major 1 congenital dyserythropoietic anemia	Mean age 31.3	M/F 1/3
Cohort 3 (16 mg/kg)	4 β-thalassemia major	Mean age 29.8	M/F 1/3
Cohort 4 (32 mg/kg)	1 α-thalassemia hydrops ($\alpha^0\alpha^0$) 2 β-thalassemia/hemoglobin E 1 sickle cell disease	Mean age 23.8	M/F 0/4

Study assessments

Adverse events were assessed at every clinic visit and between visits by telephone, from the first dose through to 28 days after the last dose (day 35). Clinical and laboratory parameters were monitored regularly beginning at screening and continued during clinic visits throughout the study (*Online Supplementary Table S1*). All clinical pathology determinations were performed at ACM Laboratories, Ltd. in Bristol, England. Seventeen blood samples for pharmacokinetics were collected from each patient: four pre-dose samples, one each on days 1, 3, 6 and 7; nine post-dose samples from 15 minutes to 8 hours after dosing on day 7; and four single samples, one each on days 8, 9, 10 and 15. Urine was collected in pooled intervals of 0-4, 4-8, and 8-24 hours after the last dose on day 7 to assess the excretion of FBS0701 and iron.

Pharmacokinetic analysis

Actual blood sampling and urine collection times were used in all pharmacokinetic analyses. Per protocol times were used to calculate mean plasma concentrations for graphical displays. A formally validated bioanalytical method for quantifying total FBS0701 in human plasma and urine was developed. The drug was extracted from plasma using solid phase extraction and analyzed using high performance liquid chromatography with tandem mass spectrometric determination, and d_3-FBS0701 as an internal standard. The method was used to measure drug (MW 400) over the range of 5 to 2000 ng/mL (12.5-5000 nM) and at sample dilutions of 20- to 50-fold. Because of the low pH, the chromatographic eluent displaces all iron from chelate complexes and thus the bioanalytical method provides total drug concentration and does not differentiate drug bound to iron from unbound drug; a method to make such a distinction is currently under development.

Pharmacokinetic parameters for FBS0701 were estimated using non-compartmental analysis. Only plasma and urine concentrations greater than the lower limits of quantitation (LOQ, 5 ng/mL in plasma, 5 ng/mL in urine) for the assays were used in the pharmacokinetic analysis. The following pharmacokinetic parameters for FBS0701 were determined from plasma concentration and urinary excretion data: the area under the plasma concentration *versus* time curve over the 24-hour time dosing interval (AUC_{0-24}); the maximum observed plasma concentration (C_{max}); the time to maximum plasma concentration (t_{max}); the terminal elimination rate constant (λz) and half-life ($t_{½}$); the apparent total plasma clearance of drug after oral administration (CL/F); the apparent volume of distribution during terminal phase after oral administration (Vz/F);

Table 2. Pharmacokinetic parameters*.

Parameter*	Dose 3 mg/kg/day	8 mg/kg/day	16 mg/kg/day	32 mg/kg/day
C_{max} (ng/mL)	5,910±2,298 (4)	15,000±4,439 (4)	38,225±3,947 (4)	68,250±27,519 (4)
T_{max} (h)	1.31 (4)	1.18 (4)	1.00 (4)	1.49 (4)
AUC(0-24) (h×ng/mL)	19,476±11,327 (4)	44,916±30,751 (4)	92,261±36,560 (4)	157,577±43,484 (4)
λz (h^{-1})	0.0655±0.0606 (4)	0.0424±0.0249 (4)	0.0421±0.0246 (4)	0.0381±0.0091 (2)
$t_{1/2}$ (h)	16.2±8.32 (4)	20.9±11.3 (4)	21.3±11.8 (4)	18.7±4.48 (2)
CL/F (mL/min)	162±85.0 (4)	225±142 (4)	206±78.4 (4)	172±60.1 (4)
Vz/F (L)	185±84.1 (4)	311±137 (4)	339±166 (4)	214±2.94 (2)
Ue(0-24) (mg)	66.1±24.0 (4)	201±68.1 (4)	402±103 (4)	641±208 (4)
Fe(0-24) (% Dose)	44.0±16.0 (4)	47.4±13.8 (4)	39.2±5.86 (4)	43.1±15.8 (4)
CLr (mL/min)	75.2±46.8 (4)	105±62.9 (4)	83.2±36.5 (4)	73.6±37.8 (4)

Values are reported as arithmetic mean ± standard deviation except T_{max} for which the median is reported.

the fraction of the oral dose excreted into urine (Ue); and renal clearance (CLr).

Statistical analysis

Plasma concentrations, blood sampling times, urine concentrations, urine volumes, and the amount excreted in each interval, and pharmacokinetic parameters were listed by dose group and patient. Plasma concentrations and pharmacokinetic parameters were summarized by dose group using descriptive statistics: linearity with respect to C_{max} and $AUC_{(0-24)}$ was assessed using the power model, i.e. $P = a \times Dose^{b}$, where P represents the parameter and *a* and *b* are constants. A log-log plot of *P versus* Dose is linear and a value of *b* of ≈1 indicates linearity. The equation was fitted to the individual patients' data using non-linear least squares regression. Parameters were compared among doses using descriptive statistics. Due to the small numbers of patients per panel, no formal statistical analyses were done.

Adverse events

Adverse events in this study were defined as any unexpected, unfavorable, harmful, or pathological change in a patient as indicated by physical signs or symptoms including intercurrent illnesses or injuries and/or clinically significant laboratory abnormalities occurring over the course of the 7-day treatment and 28-day post-treatment period. A serious adverse event was defined as an event that was fatal or life-threatening, requiring hospitalization, surgery, or resulting in a persistent disability. All adverse events and serious adverse events were assessed for their possible relationship to the study drug.

Results

Patients and study completion

Sixteen patients were enrolled and all completed the study. The primary hematological diagnoses and demographics are listed in Table 1. Additional demographic data and the patients' medical history are provided in *Online Supplementary Tables S2 and S3*).

Pharmacokinetics

The mean pre-dose plasma concentrations were relatively constant from day 3 through day 8 (*Online Supplementary Figure S1*), suggesting that steady-state had been reached by day 3, consistent with the mean $t_{½}$ (16.2 to 21.3 h; Table 2) and the 24-hour dosing interval.

The mean plasma FBS0701 concentrations after the last dose on day 7 are illustrated on linear axes in Figure 1 (0 to 24 h) and in semi-logarithmic axes in Figure 2 (0 to 192 h). Plasma concentrations increased in a dose-related manner (Figure 1) and decayed at essentially the same rate after all four doses (Figure 2) demonstrating linear pharmacokinetics. Mean values for C_{max} and $AUC_{(0-24)}$ also increased in a dose-proportional manner (Table 2) and log-log plots of the mean C_{max} and $AUC_{(0-24)}$ (Figure 3) *versus* dose were reasonably linear with slopes whose approximate 95% confidence intervals included 1.0, providing further evidence of linear pharmacokinetics. The median values for T_{max} ranged from 1.00 to 1.49 h and were not dose-dependent (Table 2). There were no dose-related trends in either CL/F or Vz/F and the mean elimination half-life, $t_{½}$, was independent of dose (Table 2).

The urinary recovery of FBS0701 was comparable across the four cohorts with the mean recovery ranging from 39.2% to 47.4% of the dose and mean renal clearance from 73.6 to 105 mL/min (Table 2). As the protein binding of FBS0701 is 85%, filtration clearance, estimated as the unbound fraction, 0.15, times the glomerular filtration rate, about 120 mL/min, would be approximately 18 mL/min. As CLr is about 5-fold greater than the filtration clearance, active tubular secretion is likely a substantial component of the renal excretion of FBS0701. There was, however, no evidence of saturation of renal clearance at the systemic exposures observed in this study.

Pharmacodynamics

In this study, urine was collected for 24 h following last administration of FBS0701 on day 7. The concentration of urinary iron was measured in three aliquots: 0-4 h, 4-8 h and 8-24 h. Iron was detected in the urine of all patients administered FBS0701 but amounts were low and no significant dose-dependent trends in urinary iron excretion were evident (*data not shown*).

Safety

All 16 patients who received FBS0701 were included in the safety analysis. The safety and tolerability of each dose level was reviewed by the Principal Investigator (EN) and the FerroKin Medical Monitor (HYR) prior to dose escalation. Adverse events, vital sign measurements, ECG results,

and laboratory assessments were reviewed to evaluate the safety and tolerability of each dose level of FBS0701. Treatment-emergent adverse events were reported by 14 of the 16 patients; Table 3 lists those adverse events possibly or probably related to FBS0701. A complete list of all the adverse events is given in *Online Supplementary Table S4*. There was no evidence to suggest a relationship between dose-escalation and the intensity or causality of adverse events. Twenty-four adverse events were mild in intensity and seven adverse events were moderate in intensity. There was one serious adverse event (*Online Supplementary Table S4*). Patient 0501 with sickle cell disease was admitted to the hospital for sickle cell pain 20 days after the last dose of FBS0701. The 3-day hospital course was uncomplicated and the patient was discharged in her baseline condition. The serious adverse event was deemed unrelated to FBS0701 by the site Principal Investigator. All adverse events with the exception of sciatica have resolved without any further medical intervention or follow-up. There were no deaths and no withdrawals from the study due to adverse events.

There were no statistically significant changes in biochemical, hematologic or urinary parameters, nor any deemed clinically significant or adverse. Specifically, there were no changes in serum creatinine, blood urea nitrogen or urinalysis from pre-dose to day 7 or to day 15. There were no changes in vital signs, physical examination, or ECG

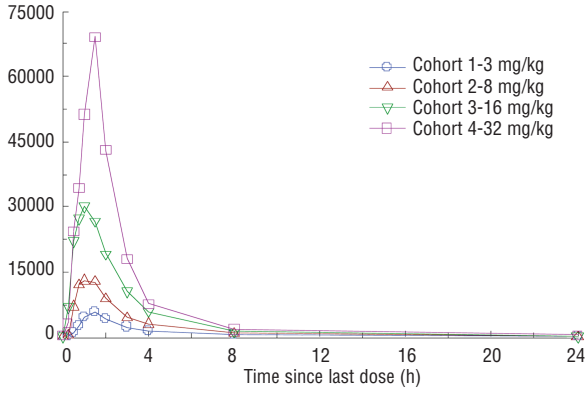

Figure 1. Mean plasma concentrations of FBS0701 after oral administration on day 7 of 3, 8, 16, or 32 mg/kg/day to patients with transfusional iron overload — linear axes, 0-24 hours.

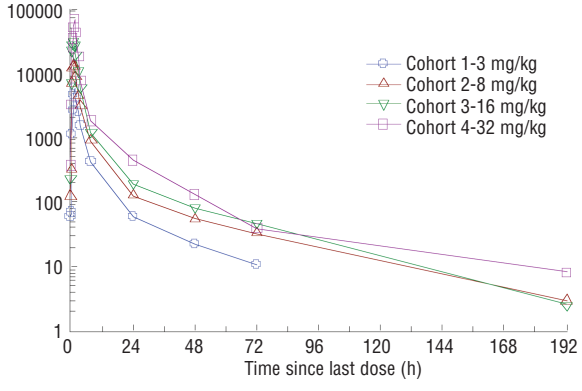

Figure 2. Mean plasma concentrations of FBS0701 after oral administration on day 7 of 3, 8, 16, or 32 mg/kg/day to patients with transfusional iron overload — log- linear axes, 0-192 hours.

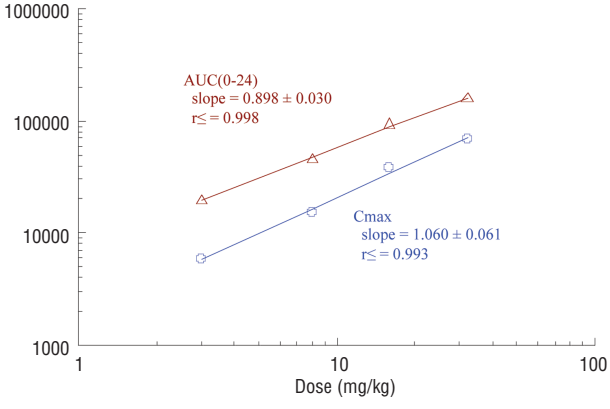

Figure 3. Relationship between the mean C_{max} and $AUC_{(0\text{-}24)}$ of FBS0701 after oral administration on day 7 of 3, 8, 16, or 32 mg/kg/day to patients with transfusional iron overload.

Table 3. Adverse events possibly or probably related to FBS0701.

Patient ID	Adverse event	Intensity	Outcome	Causality	Serious adverse event	Dose of FBS0701 in mg/kg
603	Flatulence	Mild	Resolved	Possibly related	No	3
603	Headache	Mild	Resolved	Possibly related	No	3
102	Stomach ache	Mild	Resolved	Probably related	No	8
605	Abdominal warmth	Mild	Resolved	Possibly related	No	8
701	Urine color change	Mild	Resolved	Possibly related	No	16
702	Urine color change	Mild	Resolved	Possibly related	No	16
703	Urine color change	Mild	Resolved	Possibly related	No	32
704	Urine color change	Mild	Resolved	Possibly related	No	32
704	Headache	Mild	Resolved	Possibly related	No	32
802	Urine color change	Mild	Resolved	Possibly related	No	32
802	Headache	Mild	Resolved	Possibly related	No	32
802	Pruritus	Mild	Resolved	Possibly related	No	32
802	Flatulence	Mild	Resolved	Possibly related	No	32
802	ECG change	Mild	Resolved	Possibly related	No	32

findings from pre-dose values deemed adverse with the exception of one patient with a change in QTc interval on day 7. This finding was in a fourth cohort patient (802) with sickle cell disease with a baseline QTc interval of 442 ms who was observed 4 h after the last dose on day 7 to have a QTc interval of 462 ms without other ECG changes or clinical symptoms. A repeat ECG on day 17, 10 days after her last dose of FBS0701 showed a QTc interval of 455 ms. This observation was not unexpected in a patient with sickle cell disease.[8]

Discussion

In this first multiple-dose study of FBS0701 in transfusionally iron-overloaded patients, analysis of the plasma concentration and urinary excretion data for FBS0701 after oral administration of 3 mg/kg, 8 mg/kg, 16 mg/kg, and 32 mg/kg indicated linear pharmacokinetics over that range of doses. There was no apparent dose-dependency of T_{max}, CL/F, Vz/F, or $t_{½}$. The mean elimination half-life was about 19 h and approximately 43% of the dose was recovered in the urine. CL/F appears to be directly related to body weight, suggesting that weight-based dosing is appropriate for FBS0701.

The $t_{1/2}$ of 19.2 hours suggests that once-daily dosing is feasible. Plasma levels of FBS0701 24 h following the last dose were less than 1% of C_{max}. Our assay for FBS0701 does not distinguish FBS0701 bound to iron from free drug; however, the presence of FBS0701 in the plasma at 24 h post-dosing in the third and fourth dose cohorts at concentrations of approximately 1 μM allows for the possibility that non-transferrin bound iron may be suppressed over the entire FBS0701 dosing cycle in patients taking predicted therapeutic doses of FBS0701; this assertion will need to be tested directly by measuring non-transferrin bound iron and determining the proportion of free drug to total drug in plasma at various times after dosing.

The small amount of iron found in the urine at all FBS0701 dose levels is consistent with data from rats in which 98% of iron excreted following a single dose of FBS0701 was found in bile.[5] The data in this study suggest that the fraction of the oral dose of FBS0701 not bound to iron is largely excreted renally whereas the drug:iron complex is likely to be excreted predominantly *via* the biliary route.

FBS0701 was well tolerated in the intended target population of transfusional iron-overloaded patients. The frequency or severity of adverse events did not show any dose-dependency with the exception of changes in urine color which were of no apparent clinical significance. The most common adverse events were headache (n=5), urine color change (n= 4) and flatulence (n= 2). Significantly, there were no reports of changes in the frequency or character of stools in any of the 16 patients over the 7-day dosing period as have been observed with other oral chelators over this dosing period. Although patients with moderate-to-severe renal function were excluded from the study, there were no instances in any patients of elevations in serum creatinine at any time during the 7-day dosing period or during the 28-day follow-up safety assessment period. Similarly, there were no significant changes in liver function tests.

In summary, FBS0701 was well tolerated for 7 days in iron-overloaded patients with favorable pharmacokinetics at doses likely to be therapeutic. These results warrant further safety and efficacy studies of FBS0701 in iron-overloaded populations of patients.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

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