Journal of Diabetes Science and Technology
Volume 4, Issue 5, September 2010

ORIGINAL ARTICLES

Pharmacokinetic Characterization of the Novel Pulmonary Delivery Excipient Fumaryl Diketopiperazine

Elizabeth Potocka, Ph.D.,[1] James P. Cassidy, M.S.,[1] Pamela Haworth, B.S.,[2] Douglas Heuman, M.D.,[3] Sjoerd van Marle, M.D.,[4] and Robert A. Baughman, Jr., Pharm.D., Ph.D.[1]

Abstract

Background:

Technosphere® [Bis-3,6(4-fumarylaminobutyl)-2,5-diketopiperazine (FDKP)] microparticles, the integral component of the Technosphere inhalation system, deliver drugs to the deep lung and have been used to administer insulin and glucagon-like peptide-1 via inhalation in clinical studies. Three studies were conducted to characterize FDKP pharmacokinetics, including assessments in subjects with diabetic nephropathy (DNP), in subjects with chronic liver disease (CLD), and in healthy subjects.

Methods:

An open-label, nonrandomized, two-period, fixed-sequence crossover absorption, distribution, metabolism, and excretion (ADME) study was conducted in six healthy nonsmoking men who received single intravenous and oral doses of [^{14}C]FDKP solution, with serial sampling of blood, urine, feces, and expired air. Additionally, two single-dose, open-label, parallel-design studies with 20 mg of inhaled FDKP were conducted in (1) 12 diabetic subjects with normal renal function and 24 DNP subjects and (2) 12 healthy subjects and 21 CLD subjects.

Results:

In the ADME study, >95% of the intravenous dose and <3% of the oral dose were recovered in urine, with no evidence of metabolism. No significant pharmacokinetic differences were observed between healthy subjects and CLD subjects [geometric mean (% coefficient of variation) area under the curve from time 0 to 480 minutes ($AUC_{0–480}$): 26,710 (34.8) and 31,477 (28.8) ng/ml·min, respectively]. Maximum observed drug concentration (C_{max}) and $AUC_{0–480}$ were higher in DNP subjects than in subjects with normal renal function [C_{max}: 159.9 (59.4) ng/ml versus 147.0 (44.3) ng/ml; $AUC_{0–480}$: 36,869 (47.2) ng/ml·min versus 30,474 (31.8) ng/ml·min]. None of the differences observed were considered clinically significant.

Conclusions:

Fumaryl diketopiperazine is predominantly cleared unchanged by the kidney with essentially no oral bioavailability. Technosphere is a safe delivery vehicle for medications administered via inhalation.

J Diabetes Sci Technol 2010;4(5):1164-1173

Author Affiliations: [1]Experimental Pharmacology, MannKind Corporation, Paramus, New Jersey; [2]MannKind Corporation, Valencia, California; [3]McGuire VA Medical Center, Richmond, Virginia; and [4]PRA-International, Institute for Clinical Pharmacology, Groningen, The Netherlands

Abbreviations: (ADME) absorption, distribution, metabolism, and excretion, ($AUC_{0–480}$) area under the curve from time 0 to 480 minutes, ($AUC_{0–\infty}$) area under the curve from time 0 to infinity, ($AUC_{0–t}$) area under the curve from time 0 to *t*, (BMI) body mass index, (C_{last}) last observed insulin concentration, (CLD) chronic liver disease, (C_{max}) maximum observed drug concentration, (DNP) diabetic nephropathy, (FDKP) Bis-3,6(4-fumarylaminobutyl)-2, 5-diketopiperazine, (GFR) glomerular filtration rate, (HPLC) high-performance liquid chromatography, (LSC) liquid scintillation counting, ($t_{1/2}$) terminal elimination half-life, (t_{last}) time corresponding to last observed drug concentration, (t_{max}) time to maximum observed drug concentration

Keywords: chronic liver disease, diabetic nephropathy, fumaryl diketopiperazine, inhalation, Technosphere®

Corresponding Author: Elizabeth Potocka, Ph.D., MannKind Corporation, 61 South Paramus Road, Paramus, NJ 07652; email address *epotocka@mannkindcorp.com*

Introduction

Technosphere® technology is a novel, versatile drug delivery platform that enables pulmonary administration of therapeutic agents.[1] The resulting drug product is composed of the active agent adsorbed onto the Technosphere microparticle [Bis-3,6(4-fumarylaminobutyl)-2,5-diketopiperazine (FDKP) and polysorbate 80], which utilizes a proprietary, breath-actuated inhaler for pulmonary delivery of the particles.

Fumaryl diketopiperazine is a pharmaceutical excipient that, in solution and under slightly acidic conditions, forms an array of microcrystalline plates. The plates then self-assemble into microparticles with a large surface area onto which various peptides, proteins, or other agents can be adsorbed (**Figure 1**). When dried, the resulting dry powder is filled into unit-dose cartridges, which are then used in conjunction with an inhaler.

Technosphere particles have an average diameter of 2.5 μm, with ≥90% of the particles in the respirable range for pulmonary delivery (0.5–5.8 μm), as confirmed by aerodynamic testing.[1] These characteristics enable oral inhalation of therapeutics for a wide range of disease states,[1] providing an alternative delivery route for agents currently dosed only by injection, and by making it possible to bypass hepatic first-pass metabolism by absorption directly into the arterial circulation.[2,3] This technology can accommodate therapeutics over a wide molecular size range[1] and has been used to administer insulin and glucagon-like peptide-1 by the pulmonary route in clinical studies.[3,4] A number of other agents, including oxyntomodulin and peptide YY, have been administered in nonclinical studies.[5]

Therapeutics administered via the Technosphere technology demonstrate rapid absorption, believed to be associated with rapid dissolution of the microparticles rather than any FDKP-mediated absorption enhancement. *In vitro* studies using the Calu-3 lung cell line[6] and *in vivo* rat studies (unpublished data) show that FDKP does not enhance drug absorption.

Neither *in vitro* nor preclinical *in vivo* investigations have found any evidence of a pharmacological effect of FDKP. Fumaryl diketopiperazine was evaluated in 63 *in vitro* receptor-binding assays (neurotransmitters, steroids, ion channels, secondary messengers, prostaglandin, growth factors/hormones, brain and gut peptides, and various enzymes) to evaluate potential effects. At concentrations

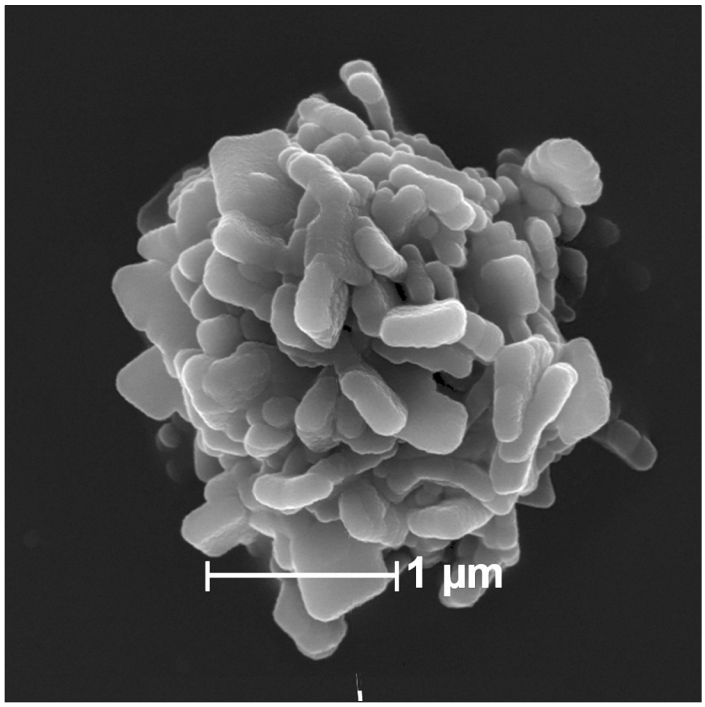

Figure 1. Scanning electron micrograph of a Technosphere® particle.

up to 100 μ*M*, FDKP did not inhibit binding of any substrate to these receptors. The biological activity of FDKP has been evaluated in comprehensive toxicology studies and in rat and dog pharmacology studies, with no observed effects on cardiac, central nervous system, pulmonary, or renal functions (unpublished data).

To characterize FDKP pharmacokinetics, an absorption, distribution, metabolism, and excretion (ADME) study was conducted in healthy human subjects utilizing ^{14}C-radiolabeled FDKP administered by both intravenous and oral routes (study 1). Subsequently, two phase 1 studies were conducted to assess FDKP pharmacokinetics in: (1) subjects with diabetic nephropathy (DNP) and diabetic subjects with normal kidney function (study 2) and (2) subjects with chronic liver disease (CLD) compared with subjects with normal hepatic function (study 3). The results of these clinical studies are reported here.

Methods

Study Conduct and Study Subject Consent

Each study protocol and the accompanying patient information were approved by local ethics committees and institutional review boards, and each subject gave written informed consent before participation. Studies were conducted in compliance with the Declaration of

Helsinki and under the current International Conference on Harmonization Guidelines for Good Clinical Practice. For studies 2 and 3, subjects were trained by site personnel on use of the MedTone® inhaler (MannKind Corporation, Danbury, CT).

Study Subject and Disease Characteristics

In all studies, subjects were nonsmokers willing to use adequate birth control. Exclusion criteria in all three studies included, but were not limited to, previous exposure to an inhaled insulin product, participation in another clinical study, or presence of clinically significant disease. In study 1, use of any medications or herbal remedies within 14 days before the first dosing (except for paracetamol) was also precluded. Complete physical examinations were performed at the screening and follow-up visits. Any clinically significant change was documented. Twelve-lead electrocardiograms were performed at protocol-specified time points with subjects in a supine position. Smoking and pulmonary status (studies 2 and 3) were reviewed at the screening, treatment, and follow-up visits.

Study 1: Human ADME Study

This phase 1, single-dose, open-label, nonrandomized, two-period, fixed-sequence crossover study was conducted in six healthy volunteers. Participants were men ≥18 and ≤60 years of age deemed to be healthy based on screening physical examination, medical history, normal clinical chemistry and urinalysis, fasting blood glucose ≤110 mg/dl, and body mass index (BMI) ≤30 kg/m^2 (nonobese).

The study consisted of four clinic visits, with study treatment administration of ^{14}C-labeled FDKP (see **Figure 2** for FDKP structure) on visits 2 and 3. Subjects underwent serial sampling of blood, urine, feces, and expired air at both treatment visits. During visit 2, all eligibility criteria were confirmed and subjects received a single infusion of 10 ml FDKP (3.7 MBq of [^{14}C]FDKP) intravenously over 5 minutes at 2 mg/min for a total dose of 10 mg FDKP. Urine and feces from all subjects were assessed daily by liquid scintillation counting ("quick counts"). If quick counts did not meet discharge criteria (<50 dpm/ml urine, <75 dpm/100 mg feces in 24-hour collection) on day 4, 24-hour urine and feces specimens were collected until criteria were met. After a minimum 2-week washout period, at visit 3, each subject received a single 100-ml oral dose of FDKP solution (0.2 mg/ml, containing 2.5 MBq of [^{14}C]FDKP). The final 20-mg dose is an approximate equivalent of the FDKP amount in the 60-unit Technosphere Insulin dose, a clinically relevant dose studied frequently in the Technosphere Insulin development program.[7–9]

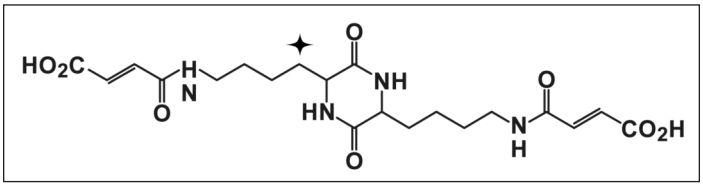

Figure 2. Structure of FDKP. The symbol indicates the position of the ^{14}C label.

All subjects remained in the clinic until day 4, and quick counts were conducted as per visit 2. Visit 4 was a follow-up that took place 7–14 days after visit 3.

Study 2: Diabetic Subjects with Mild or Moderate Diabetic Nephropathy or Normal Renal Function

This single-dose, open-label, parallel, controlled study of FDKP was conducted in diabetic subjects with normal renal function and mild or moderate DNP. The study consisted of three clinic visits: screening, treatment, and follow-up. Each subject received one 20-mg (cartridge fill weight) dose of Technosphere inhalation powder at the treatment visit. Blood and urine samples were collected serially over 24 hours postdose.

Inclusion criteria consisted of men and women ≥18 and ≤80 years of age with type 1 or type 2 diabetes confirmed by medical records and a BMI ≤37 kg/m^2. Staging of subjects with DNP was based on recommendations of the National Kidney Foundation Disease Outcomes Quality Initiative clinical practice guidelines.[10] Mild DNP was defined as a glomerular filtration rate (GFR) of 60–89 ml/min/1.73 m^2 and microalbuminuria (≥17 mg/g in men, ≥25 mg/g in women). Moderate DNP was defined as a GFR of 30–59 ml/min/1.73 m^2 and microalbuminuria (≥17 mg/g in men, ≥25 mg/g in women). Normal renal function required a GFR ≥90 ml/min/1.73 m^2 without microalbuminuria (<17 mg/g in men, <25 mg/g in women).

Study 3: Subjects with Mild or Moderate Chronic Liver Disease or Normal Liver Function

This single-dose, open-label, parallel, controlled study was conducted in nondiabetic subjects with normal liver function and mild or moderate CLD. This study consisted of screening, treatment, and follow-up visits and 20 mg of Technosphere inhalation powder (cartridge fill weight) administered at the treatment visit. Blood and urine samples were collected serially over 24 hours postdose.

Inclusion criteria consisted of men and women ≥18 and ≤80 years of age with a BMI ≤35 kg/m^2. Chronic liver disease staging was based on the Child–Pugh score with recommendations of the American Association for the Study of Liver Diseases practice guidelines.[11] Mild CLD was defined as Child–Pugh class A (5–6 points), and moderate CLD was defined as Child–Pugh class B (7–9 points). Disease history included histopathological confirmation or clinical imaging and laboratory findings. Subjects without CLD had an absence of clinical evidence of liver disease, negative hepatitis B and hepatitis C serology, and normal liver function tests.

Bioanalytical Methods

Serial blood samples were collected into tubes on ice. In study 1, plasma was analyzed for radioactivity by liquid scintillation counting (LSC). Urine and feces were collected and pooled for specified timed intervals. Urine samples were analyzed for radioactivity (LSC), whereas the radioactivity in feces was measured via sample combustion (Packard 307 sample oxidizer). Expired air was assessed for $^{14}CO_2$ with hyamine hydroxide as a trapping solution and phenolphthalein in ethanol as an indicator. Subjects exhaled via a tube placed into a vial containing the solution until the solution became colorless (≈2 minutes), indicating neutralization by CO_2. Vials were stored at 4 °C until assay on site for ^{14}C radioactivity by LSC. For studies 2 and 3, samples for the FDKP assay were centrifuged at 4 °C within 1 hour of collection and frozen until FDKP quantification by liquid chromatography with tandem mass spectrometry.

Metabolites in Urine and Feces

Urine and plasma samples with a level of radioactivity >4000 DPM/ml were analyzed by high-performance liquid chromatography (HPLC) with radiochemical detection (**Table 1**), a method sensitive enough to separate the *cis* and *trans* FDKP isomers. The injection volume was ≤75 µl to prevent overloading of the column.

Pharmacokinetic Parameters

Pharmacokinetic parameters included maximum plasma drug concentration (C_{max}), time to C_{max} (t_{max}), and area under the curve from time 0 to t (AUC_{0-t}, calculated by the linear-trapezoidal method). Terminal elimination half-life ($t_{1/2}$) was calculated as $\ln(2)/\lambda_z$, where λ_z is the terminal elimination rate constant estimated from log-linear regression analysis of the terminal elimination phase of the concentration–time profile, and area under the curve from time 0 to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-tlast} + C_{last}/\lambda_z$, where C_{last} is the last observed drug concentration and t_{last} is the time corresponding to C_{last}. In study 1, additional parameters included mass balance, calculated as the sum of the amount of ^{14}C radioactivity excreted in urine, feces, and expired air divided by the amount of radioactivity dosed. Pharmacokinetic analyses were performed using WinNonlin Professional, version 5.0.1 or higher (Pharsight, Inc., Mountain View, CA), and summary statistics and statistical analyses were performed using SAS®, version 8.2 or higher (SAS Institute, Inc., Cary, NC).

Table 1.
High-Performance Liquid Chromatography: Conditions for Profiling Serum and Urine Samples[a]

Column	Luna phenyl-hexyl 3-µm packing, 150 × 3.0 mm ID		
Guard cartridge	Phenyl, 3 µm, 4 × 2 mm ID		
Mobile phases	A 1000:1 (v/v) water:TFA B 900:100:1 (v/v) MeOH:THF:TFA		
Mobile phase gradient	Time (min)	%A	%B
	0	95	5
	35	65	35
	37	5	95
	40	5	95
	41	95	5
Flow rate	0.300 ml/min		

[a] ID, inside diameter; TFA, trifluoroacetic acid; MeOH, methanol; THF, tetrahydrofuran.

Results

Study 1: Human ADME

Four of the six enrolled subjects completed the study. Two subjects were withdrawn after the intravenous dose but before oral dosing (prior to starting visit 3) due to elevated levels of urinary cotinine (>100 ng/ml), in violation of inclusion criteria (i.e., nonsmoking status). Hence, the pharmacokinetic analysis included six healthy subjects following intravenous dosing and four subjects following oral dosing (**Table 2**).

Following intravenous administration, >90% of total radioactivity elimination occurred within 8 hours of dosing (**Table 3**, **Figure 3A**). An average of 97.2% of total radioactivity was recovered in the urine and 1.65% was recovered in the feces. Analyses of urine and dosing materials by HPLC demonstrated that all radioactivity peaks detected in urine were present in the dosing material in the same proportions. There was no evidence

of metabolism following intravenous dosing. The radiochromatogram from a representative urine sample (**Figure 4**) shows the *cis* and *trans* FDKP isomers, with no other radioactivity peaks evident.

Following oral dosing, >90% of radiolabel was eliminated within 48 hours postdose, with an average of 2.45% of total radioactivity recovered in the urine and approximately 95.1% recovered in the feces (**Table 3**, **Figure 3B**). A negligible fraction of the dose was recovered in expired air (0.03% of the dose). Pharmacokinetic parameters and ^{14}C radioactivity of FDKP in plasma are summarized in **Table 4** and **Figures 5** and **6**.

Study 2: Diabetic Subjects with Diabetic Nephropathy and Normal Renal Function

All 36 enrolled subjects completed the study (**Table 5**). No significant differences were noted in subject characteristics among the three renal function groups, with the exception of race; the majority of subjects in the mild DNP group were of Hispanic origin. Given the small number of subjects, the determination of racial differences on FDKP pharmacokinetics was not possible;

Table 2.
Mean (SD[a]) Demographics and Baseline Characteristics of Subjects in Study 1

	Intravenous dose population (n = 6)	Oral dose population (n = 4)
Age, years (SD)	29.8 (14.9)	34.0 (17.2)
Weight, kg (SD)	77.7 (10.5)	77.2 (13.6)
Height, cm (SD)	180.3 (12.7)	177.3 (15.2)
BMI, kg/m^2 (SD)	23.9 (1.2)	24.5 (1.0)
Race, n (%)		
Caucasian	5 (83.3)	3 (75.0)
Asian	1 (16.7)	1 (25.0)

[a] Standard deviation.

Table 3.
Summary of ^{14}C Radioactivity Pharmacokinetic Parameters in Urine, Feces, and Expired Air: Study 1

Parameter	10-mg IV dose (n = 6)			20-mg oral dose (n = 4[a])		
	Urine	Feces	Expired air	Urine	Feces	Expired air
Ae,[b] µg	9720	165.0	0	490.0	19,030	5
Fe, %	97.2	1.65	0	2.45	95.1	0.03
Mass balance, % (SD)	98.9 (2.1)			97.6 (1.5)		

[a] Two subjects did not receive oral dose of study drug because they were withdrawn from the study.
[b] Ae, total amount of ^{14}C radioactivity excreted in urine or feces, or estimated total amount of ^{14}C radioactivity excreted in expired air; Fe, percentage of dose of ^{14}C radioactivity excreted in urine, feces, or expired air; IV, intravenous; SD, standard deviation.

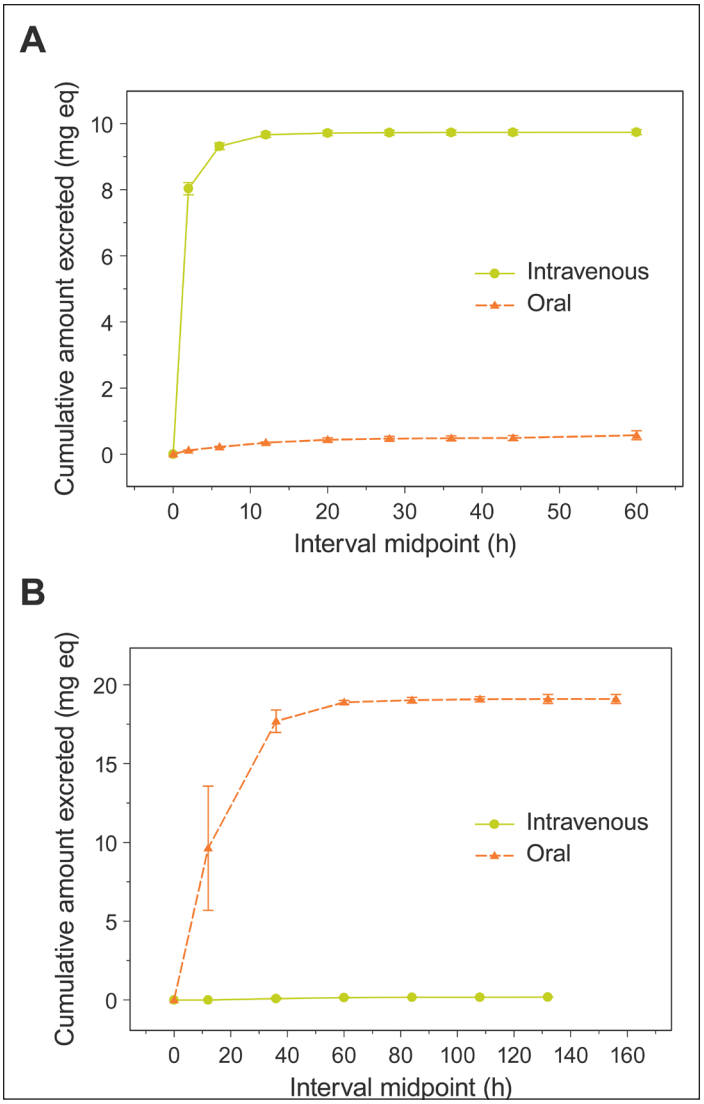

Figure 3. Mean (±SE) cumulative excretion of total radioactivity in **(A)** urine and **(B)** feces following a 10-mg intravenous and a 20-mg oral FDKP dose.

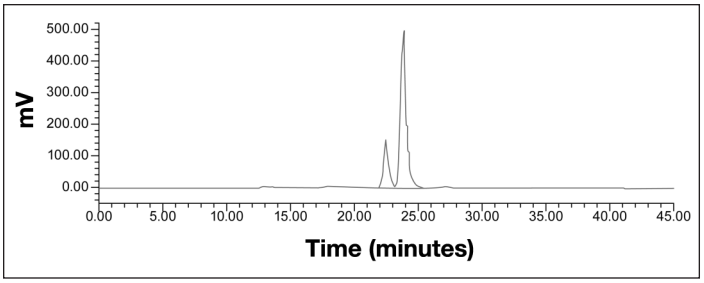

Figure 4. Representative radiochromatogram of a 0- to 4-hour urine sample following intravenous administration of ^{14}C-labeled FDKP showing the *cis* (first peak) and *trans* (second peak) isomers of FDKP.

however, there is no expectation that FDKP clearance is affected by race, as there were no obvious differences in FDKP parameters in the four racial groups within each renal function category.

Fumaryl diketopiperazine exposure [FDKP area under the curve from time 0 to 480 minutes (AUC_{0-480})] was approximately 18 and 25% higher in subjects with mild and moderate DNP, respectively, compared with subjects with normal renal function (**Table 6**, **Figure 7**). Fumaryl diketopiperazine $t_{1/2}$ was similar in subjects with normal renal function and those with mild DNP and was ≈80 minutes longer in subjects with moderate DNP. This difference in $t_{1/2}$ resulted in a more noticeable difference in exposure over a longer sampling period in subjects with moderate DNP, with an approximate 52% greater exposure when $AUC_{0-\infty}$ was compared with subjects with normal renal function. The longer t_{max} observed in subjects with moderate DNP (30 minutes versus 12 minutes in subjects with normal renal function and 15 minutes in those with mild DNP) is most likely also attributable to the observed longer $t_{1/2}$.

Over 24 hours, urinary elimination was almost complete, with approximately 20% of the FDKP dose excreted in the urine of all subjects, with slower accumulation in

Table 4.
Summary of Pharmacokinetic Parameters and ^{14}C Radioactivity of FDKP in Plasma: Study 1

Parameter[a]	Intravenous dose (10 mg) $n = 6$	Oral dose (20 mg) $n = 4$
$AUC_{0-\infty}$, ng/ml·min	75,240 (5640)	NC
$AUC_{0-tlast}$, ng/ml·min	74,791 (5640)	5,587 (3028)
C_{max}, ng/ml	722.7 (130.5)	8.5 (1.6)
t_{max}, h	NC	6 (1, 16)
$t_{1/2}$, min	150 (24)	NC
F_{oral}	NA	0.0364 (0.0174)

[a] Parameters are presented as mean (standard deviation) except for t_{max}, which is presented as median (min, max).
$AUC_{0-tlast}$, area under the plasma concentration–time curve from time 0 to t, where t is time of last quantifiable concentration; F_{oral}, bioavailability following oral dose; NA, not applicable; NC, not calculated.

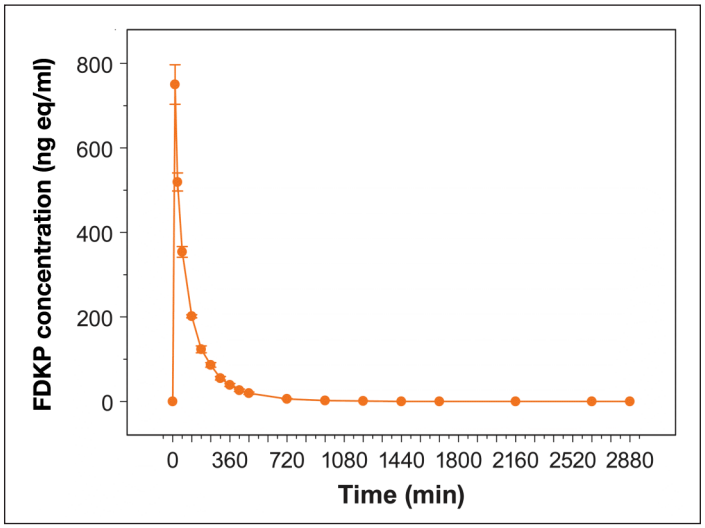

Figure 5. Mean (±SE) total radioactivity in plasma following a 10-mg FDKP intravenous dose.

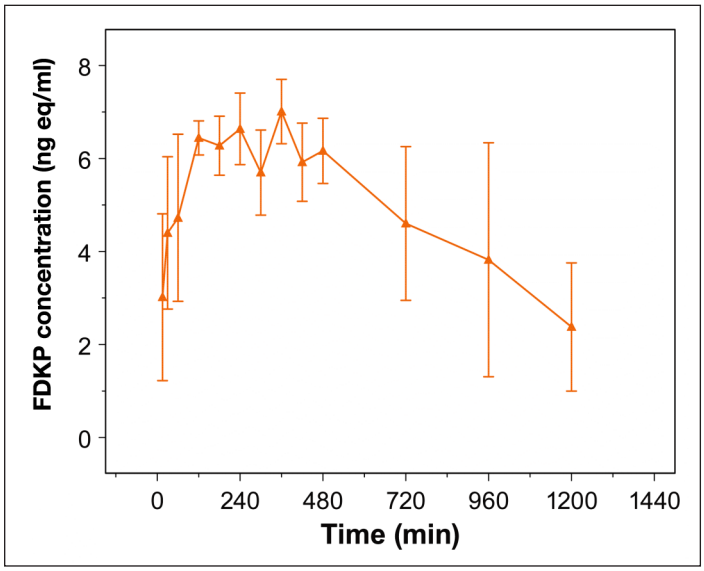

Figure 6. Mean (±SE) total radioactivity in plasma following a 20-mg FDKP oral dose (linear scale).

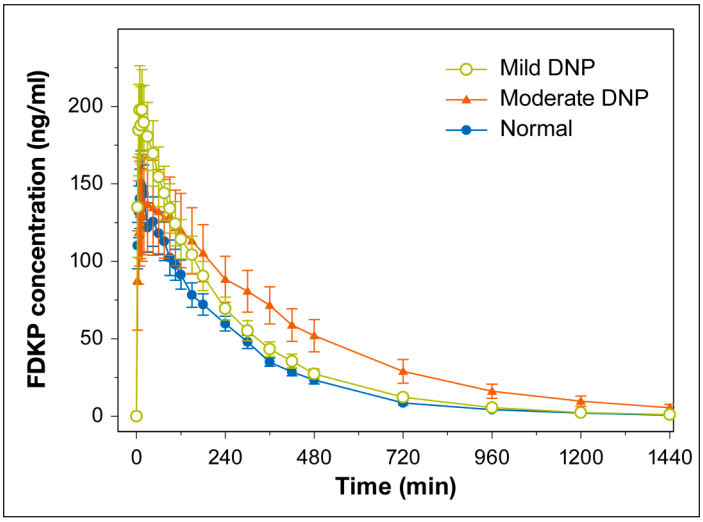

Figure 7. Mean (±SE) FDKP concentration–time profiles in subjects with diabetes and diabetic nephropathy and normal kidney function (study 2).

subjects with moderate DNP compared with the other subjects (**Figure 8**).

Study 3: Subjects with Chronic Liver Disease and Normal Liver Function

Study enrollment was stopped at 33 subjects before the target enrollment of 36 subjects was achieved (**Table 5**) due to the prolonged enrollment period necessary to identify CLD subjects. No significant differences in subject characteristics were noted among the three hepatic function groups.

Fumaryl diketopiperazine exposure (AUC_{0-480}) was higher in subjects with CLD than in those with normal liver function; however, this difference was slight (**Table 7**, **Figure 9**). No differences in FDKP C_{max} and t_{max} and $t_{1/2}$ were observed. Over 24 hours, urinary elimination was almost complete with, on average, <25% of the FDKP dose excreted in urine for all subjects (**Figure 10**). Differences in mean cumulative excretion amounts were skewed for the mild CLD group by one subject with high excretion values; this apparent difference was negated when that subject was excluded.

Table 5.
Mean (SD[a]) Demographics and Baseline Characteristics of Subjects in Studies 2 and 3

	Diabetic subjects without DNP (*n* = 12)	Mild DNP (*n* = 15)	Moderate DNP (*n* = 9)	Normal (*n* = 12)	Mild CLD (*n* = 15)	Moderate CLD (*n* = 6)
Age, years (SD)	58 (7)	63 (9)	52 (13)	51 (7)	58 (8)	58 (3)
BMI, kg/m^2 (SD)	31 (4)	29 (4)	33 (3)	28 (4)	30 (4)	28 (2)
Sex						
Male (%)	7 (58)	11 (73)	6 (67)	12	14	6
Female (%)	5 (42)	4 (27)	3 (33)	0	1	0
Weight, kg (SD)	88 (16)	80 (13)	97 (11)	92 (18)	95 (15)	88 (11)
Height, cm (SD)	167 (11)	165 (7)	170 (8)	181 (8)	178 (10)	176 (7)
Race, *n*						
Caucasian	4	2	3	9	8	3
Black	1	0	1	2	5	2
Hispanic	7	13	4	1	2	1
Other	0	0	1	0	0	0

[a] Standard deviation.

Table 6.
Geometric Mean (% Coefficient of Variation) FDKP Pharmacokinetic Parameters in Diabetic Nephropathy: Study 2

Parameter	Diabetic without DNP (*n* = 12)	Diabetic nephropathy		
		Mild (*n* = 15)	Moderate (*n* = 9)	Mild and moderate (*n* = 24)
AUC_{0-480}, ng/ml·min	30,474 (32)	36,090 (43)	38,206 (54)	36,869 (47)
$AUC_{0-\infty}$, ng/ml·min	37,137 (29)	44,168 (43)	56,393 (58)	48,406 (53)
C_{max}, ng/ml	147.0 (44)	184.1 (55)	126.4 (66)	159.9 (59)
t_{max}, min[a]	12 (3–45)	15 (3–76)	30 (9–153)	16 (3–153)
$t_{1/2}$, min	190.6 (52)	196.2 (46)	269.8 (65)	223.8 (64)
Ae_{0-1440}, mg[b]	3.7 (1.2)	3.7 (1.7)	2.8 (1.7)	3.3 (1.7)

[a] t_{max} is presented as median (range).
[b] Ae is presented as arithmetic mean (standard deviation).

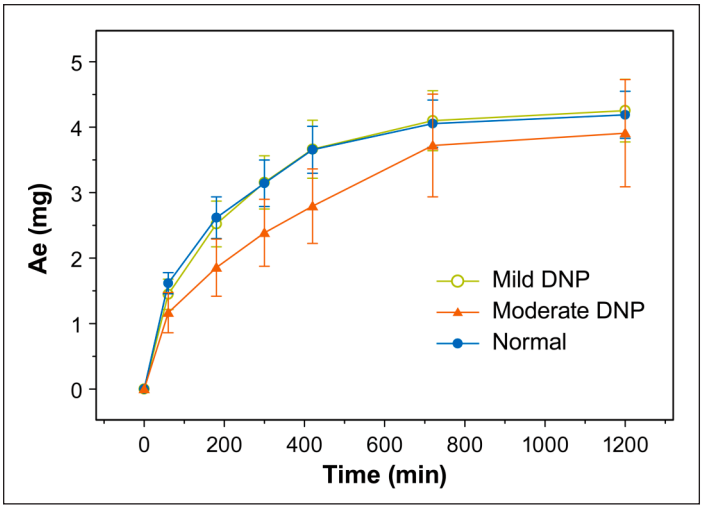

Figure 8. Mean (±SE) cumulative amount of FDKP excreted in urine in subjects with diabetes and diabetic nephropathy and normal kidney function (study 2).

Safety: All Studies

Administration of FDKP was generally well tolerated in all studies, and treatment-related adverse events were mild with no associated discontinuations. No severe adverse events or deaths occurred during these studies.

In study 1, all treatment-related adverse events were mild in intensity except for one instance of iridocyclitis of moderate intensity, not related to the study drug. Cough, the most common treatment-emergent adverse event, was reported by 19 (52.8%) subjects in study 2 and by 17 (51.5%) subjects in study 3. In both studies, all coughs were nonproductive, mild in intensity, and occurred within 10 minutes of inhalation and as a single or intermittent occurrence.

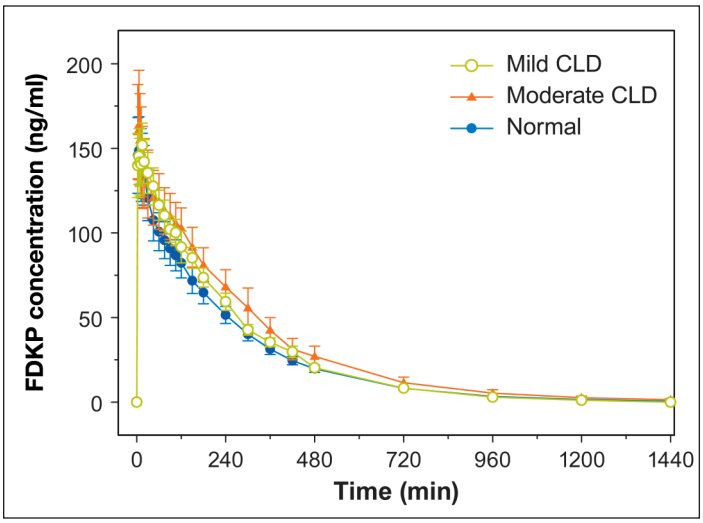

Figure 9. Mean (±SE) FDKP concentration–time profiles in subjects with normal and impaired hepatic function (study 3).

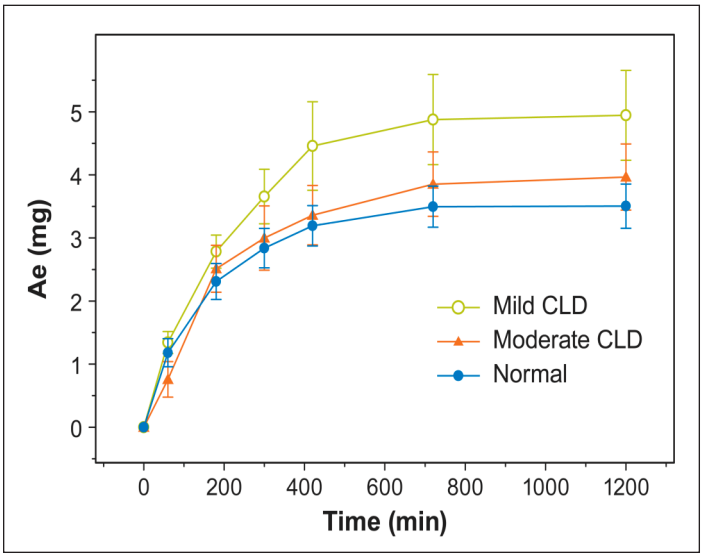

Figure 10. Mean (±SE) cumulative amount of FDKP excreted in urine (Ae) in subjects with impaired and normal hepatic function (study 3).

Table 7.
Geometric Mean (% Coefficient of Variation) FDKP Pharmacokinetic Parameters in Chronic Liver Disease: Study 3

Parameter	Normal (n = 12)	Chronic liver disease		
		Mild (n = 15)	Moderate (n = 6)	Mild and moderate (n = 21)
AUC_{0-480}, ng/ml·min	26,710 (35)	31,001 (26)	32,700 (36)	31,477 (29)
$AUC_{0-\infty}$, ng/ml·min	32,320 (33)	36,505 (26)	40,085 (41)	37,494 (32)
C_{max}, ng/ml	143.4 (49)	161.5 (35)	156.8 (42)	160.2 (36)
t_{max}, min[a]	7.5 (3–20)	6.0 (3–60)	6.0 (3–45)	6.0 (3–60)
$t_{1/2}$, min	190 (27)	173 (30)	198 (45)	180 (36)
Ae_{0-1440}, mg[b]	3.2 (1.1)	4.5 (2.7)	3.4 (1.2)	4.1 (2.4)

[a] t_{max} is presented as median.
[b] Ae is presented as arithmetic mean (standard deviation).

Discussion

The Technosphere inhalation system can be used to deliver various therapeutic agents via the pulmonary route. Fumaryl diketopiperazine, the primary component of Technosphere particles, is a novel, pharmacologically inert compound that is absorbed rapidly into the systemic circulation.[1] Because administration of Technosphere particles results in systemic FDKP exposure, the pharmacokinetic properties of this compound were characterized.

Inhaled drugs may enter the systemic circulation via two routes: (1) a fraction of the dose enters through the lung (directly into the central compartment) and (2) a fraction of the dose is swallowed. Therefore, FDKP metabolism was assessed following both intravenous and oral routes of administration to fully characterize its clearance after inhalation. Following intravenous administration, almost complete renal clearance of unchanged FDKP was observed. In the same study, FDKP demonstrated essentially no oral bioavailability; hence, the route of elimination of drug following pulmonary dosing is expected to be predominantly renal, as any swallowed drug is not expected to be systemically bioavailable at clinically relevant doses.

In studies 2 and 3, the cumulative amount of FDKP excreted in the urine after inhalation was measured following dosing. Approximately 20–23% of the dose was excreted at 24 hours in all groups studied. Because the ADME study showed almost complete urinary FDKP elimination by 8 hours following intravenous dosing and because FDKP is absorbed rapidly from the lung, this quantity was assumed to reflect FDKP bioavailability following inhalation. Absolute bioavailability was also calculated from mean FDKP $AUC_{0-\infty}$ in studies 2 and 3 and FDKP $AUC_{0-\infty}$ following intravenous administration in study 1 (24.7 and 21.5%, respectively), confirming the value determined from urinary excretion data. Fumaryl diketopiperazine bioavailability less than unity can be attributed to the fact that some of the drug product remains in the inhaler and some is swallowed during inhalation.

Differences observed in FDKP rate and extent of exposure between subjects with DNP and diabetic subjects without renal disease are consistent with the predominant renal clearance of the compound. However, differences observed were not considered clinically significant, as FDKP exposure is expected to remain within the safety margins with repeated dosing, based on the NOAEL (no observed adverse effect level) established in nonclinical studies (unpublished data) and an accumulation ratio (R = $AUC_{0-\infty}/AUC_{0-480}$, based on geometric means of AUCs) of 1.22 (normal renal function) and 1.31 (mild and moderate DNP) with three-times-per-day dosing. When data from subjects with moderate DNP were examined, an approximate 50% increase in FDKP exposure (based on $AUC_{0-\infty}$) was observed compared with diabetic subjects without renal impairment. This finding was without expected clinical implications due to the lack of FDKP pharmacological activity following extensive *in vitro*, preclinical, and toxicological studies.

Interestingly, although inhaled FDKP is cleared predominantly by the kidneys, study 3 showed an increase in FDKP exposure in subjects with mild and moderate CLD compared with healthy subjects. It is well established that decreased renal function occurs in subjects with hepatic disease[12,13] and that reduced renal excretion of a number of drugs cleared unchanged in urine (i.e., furosemide, bumetanide, cimetidine, and ranitidine) in subjects with hepatic impairment has been reported.[13] Therefore, it is likely that the slightly elevated FDKP exposure in subjects with CLD is linked to impaired renal clearance associated with reduced hepatic function. However, this small, relative increase in FDKP exposure was not considered clinically significant.

In studies 2 and 3, pharmacokinetic data following inhalation were available from subjects without CLD or DNP. Most parameters were comparable between these two groups, with somewhat higher exposure (C_{max}) and later t_{max} in study 2. This difference is most likely attributed to the difference in populations and the potential effect of the diabetic state on renal clearance of FDKP in study 2.

In studies 2 and 3, cough was the common treatment-emergent adverse event. The cough was mild and was observed as a single or intermittent occurrence. This finding was not surprising, as cough is often associated with the inhalation of dry powder.[14]

Conclusions

In an ADME study conducted in healthy male volunteers, >95% of the intravenous FDKP dose and <3% of the oral FDKP dose were recovered unchanged in urine. Oral bioavailability was negligible, and no evidence of

metabolism was observed. FDKP was well tolerated in all populations studied. The increase in overall exposure due to mild or moderately impaired renal function was not clinically significant. Fumaryl diketopiperazine (Technosphere) is a safe delivery vehicle for medications administered via inhalation.

Funding:

Studies discussed in this article were funded by MannKind Corporation, Valencia, CA.

Acknowledgments:

We acknowledge the following principal investigators: Robert J. Schwab, M.D., Qualia Clinical Research, Omaha, NE; Sherwyn L. Schwartz, M.D., Cetero Research, Inc., San Antonio, TX; Ernesto Fuentes, M.D., Elite Research Institute, Miami, FL; Krishna K. Pudi, M.D., Upstate Pharmaceutical Research, Greenville, SC; and Richard A. Preston, M.D., M.B.A., Division of Clinical Pharmacology, University of Miami, West Miami, FL.

Disclosure:

Dr. Potocka, Mr. Cassidy, Ms. Haworth, and Dr. Baughman are employed by MannKind Corporation. Drs. Heuman and Van Marle were principal investigators. Dr. Heuman has received research grant support from MannKind Corporation.

References:

1. Leone-Bay A, Grant M. Technosphere/insulin: mimicking endogenous insulin release. 2nd ed. In: Rathbone MJ, Hadgraft M, Roberts M, Lane M, editors. Modified release drug delivery technology. Vol 2. New York: Informa Healthcare USA, Inc.; 2008. p. 673-9.
2. Rave K, Potocka E, Boss AH, Marino M, Costello D, Chen R. Pharmacokinetics and linear exposure of AFRESA compared with the subcutaneous injection of regular human insulin. Diabetes Obes Metab. 2009;11(7):715-20.
3. Marino MT, Costello D, Baughman R, Boss A, Cassidy J, Damico C, van Marle S, van Vliet A, Richardson PC. Pharmacokinetics and pharmacodynamics of inhaled GLP-1 (MKC253): proof-of-concept studies in healthy normal volunteers and in patients with type 2 diabetes. Clin Pharmacol Ther. 2010;88(2):243-50.
4. Boss AH, Yu W, Ellerman K. Prandial insulin: is inhaled enough? Drug Dev Res. 2008;69:138-42.
5. Grant ML, Greene S, Stowell GW, Daniels S, Smithson A, Villanueva S, Leone-Bay A. Mimicking endogenous peptide secretion by inhalation [poster]. Presented at: 21st American Peptide Symposium; June 7-12, 2009; Bloomington, IN.
6. Angelo R, Rousseau K, Grant M, Leone-Bay A, Richardson P. Technosphere insulin: defining the role of Technosphere particles at the cellular level. J Diabetes Sci Technol. 2009;3(3):545-54.
7. Phillips M, Amin N, Boss AH, Richardson P. Pulmonary functions (over 2 years) in diabetic subjects treated with Technosphere insulin or usual antidiabetic treatment [abstract]. Diabetologia. 2009;52(Suppl 1):S361. Abstract 920.
8. Rosenstock J, Lorber DL, Gnudi L, Howard CP, Bilheimer DW, Chang PC, Petrucci RE, Boss AH, Richardson PC. Prandial inhaled insulin plus basal insulin glargine versus twice daily biaspart insulin for type 2 diabetes: a multicentre randomised trial. Lancet. 2010;375(9753):2244-53.
9. Peyrot M, Rubin RR. Patient reported outcomes in adults with type 2 diabetes using mealtime insulin monomer human (rDNA origin) inhalation powder (Technosphere insulin inhalation powder) or metformin + secretagogue or both [abstract]. Diabetologia. 2009;52(Suppl 1):S396. Abstract 1009.
10. National Kidney Foundation. Part 5. Evaluation of laboratory measurements for clinical assessment of kidney disease. Am J Kidney Dis. 2002;39(2 Suppl 1):S76-10.
11. Murray KF, Carithers RL Jr; AASLD. AASLD practice guidelines: evaluation of the patient for liver transplantation. Hepatology. 2005;41(6):1407-32.
12. Morgan DJ, McLean AJ. Clinical pharmacokinetic and pharmacodynamic considerations in patients with liver disease. An update. Clin Pharmacokinet. 1995;29(5):370-91.
13. Verbeeck RK. Pharmacokinetics and dosage adjustment in patients with hepatic dysfunction. Eur J Clin Pharmacol. 2008;64(12):1147-61.
14. Siekmeier R, Scheuch G. Inhaled insulin--does it become reality? J Physiol Pharmacol. 2008;59 (Suppl 6):81-113.