Initial Clinical Trial Results of a Real-Time Point-of-Care Glomerular Filtration Rate Measurement Utilizing a Novel Fluorescent Tracer Agent

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ABSTRACT

The fluorescent tracer agent, designated MB-102, with chemical name 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine has been engineered to have photophysical properties and clearance properties for use as a direct measure of glomerular filtration rate (GFR). Post intravenous administration, the clearance of this agent may be monitored noninvasively by transdermal fluorescence. Thus its use for real-time point-of-care GFR determination should have great impact in the ICU, as well as in many other settings. In this poster we present results of a clinical trial, conducted on subjects recruited to have normal renal function, which is the first in a series of clinical trials necessary to obtain regulatory clearance from the FDA. We report herein the comparison of plasma pharmacokinetics between MB-102 and the known standard exogenous GFR agent iohexol. A prototype noninvasive fluorescence detection device was employed to simultaneously measure the transdermal fluorescence from MB-102 in these same subjects. Blood samples over a period of 12 hours were collected from each subject to assess pharmacokinetic parameters including clearance. Urine samples were collected over this same period to assess percent injected dose recovered in the urine. Transdermal fluorescence was measured for over 4 hours post administration of the agent to assess correlation with the plasma pharmacokinetics.

BACKGROUND

Measurement of glomerular filtration rate (GFR) is widely accepted as the most reliable measure of renal function (National Kidney Foundation, 2002). As a result there is a growing medical need for determining accurate real-time GFR for minimizing the risk of kidney injury due to acute and chronic conditions. The optimum measure of GFR is by the use of exogenous tracer agents. However this methodology requires several blood draws as a function of time and subsequent sophisticated laboratory analysis to measure tracer agent concentration in each blood draw needed for GFR determination. Hence use of these exogenous tracer agents is not amenable to the bedside for point-of-care application, and are mainly employed for research purposes (Endre et al., 2011).

To overcome the deficiencies of the research GFR tracer agents with respect to bedside point-of-care application, effort has been recently directed at employing exogenous fluorescent agents that can be detected transdermally (Chinen et al., 2008; Rabito et al., 2005; Schock-Kusch et al., 2009; Yu et al., 2007). This methodology would combine the optimum measurement of an exogenous tracer agent with point-of-care bedside utility. To this end, we have synthesized MB-102, a fluorescent tracer that has exhibited characteristics essential for accurate real-time measurement of GFR (Poreddy et al., 2012; Rajagopalan et al., 2011).

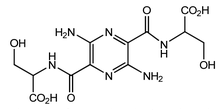

Figure 1: Chemical structure of MB-102

Pictured below is a schematic of our noninvasive transdermal measurement methodology as applied to animal models (Poreddy et al., 2012; Rajagopalan et al., 2011), which has been adapted for use in our human clinical studies.

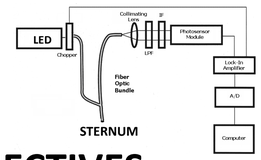

Figure 2: Schematic of noninvasive transdermal measurement methodology

OBJECTIVES

1. Simultaneously determine the plasma pharmacokinetics of MB-102 and iohexol in adult subjects with normal renal function, and compare the GFR deduced from the MB-102 data with that deduced from the iohexol data.
2. Monitor the safety and tolerability of MB-102 in healthy adult volunteers.
3. Noninvasively measure the transdermal fluorescence of MB-102 and compare this fluorescence pharmacokinetics with the MB-102 plasma pharmacokinetics.
4. Measure percent administered dose appearing in the urine for each agent.

METHODS

Plasma Pharmacokinetics:
Blood samples were collected for 12 hours following administration of MB-102 in 32 subjects with normal renal function (as determined by baseline serum creatinine measurement). Plasma concentrations of MB-102 and iohexol were measured, and standard pharmacokinetic software (Phoenix WinNonlin (Certara, Princeton, NJ)) was employed to determine clearance (and hence GFR) from the concentration versus time data for each agent.

Fluorescence Pharmacokinetics:
The noninvasive fluorescence detection instrument has a 450 nm LED source and photomodule detectors with filters to collect fluorescent light around the 560 nm peak emission of MB-102 (Figure 3a). A commercial bifurcated fiber optic bundle delivered the excitation light and collected the emission fluorescence light (Figure 3b). A prism on the common end of the bundle and the use of a StatLock (Bard Medical, Covington, GA) adhesive catheter stabilization device enabled direct coupling to the skin (Figure 3c). Four fiber optic bundles were simultaneously employed. The body positions for measurement were forehead, sternum, upper arm, and trunk (side of body).

Figure 3a

Figure 3b

Figure 3c

Percent administered dose in urine:
Total urine was collected via jug for 12 hours following administration of MB-102 in 32 subjects with normal renal function (as determined by baseline serum creatinine measurement). Concentration s of MB-102 and iohexol were measured in each subject sample, and total concentration with respect to administered dose was calculated for each subject.

RESULTS AND DISCUSSION

PLASMA PHARMACOKINETICS

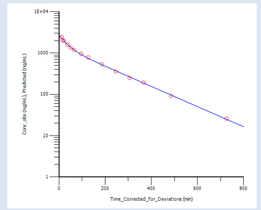

Figure 4: Concentration of MB-102 in plasma over time for Subject 3 of Pilot 1A

Data was fit to a two-compartment model. The first is the vascular-to-tissue distribution and equilibrium occurring upon administration, and the second is the elimination phase resulting from renal excretion only. The terminal half-life is ~123 minutes and the clearance (GFR) is 107 mL/min. Red circles are measured values. Blue line is the fit.

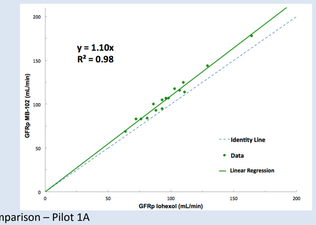

Figure 5: GFR Comparison – Pilot 1A

All 16 subjects were dosed at 1 µmol/kg. Glomerular Filtration Rate (GFR) from the time-dependent plasma concentration of MB-102 is plotted against the GFR from the time-dependent plasma concentration of iohexol. A linear regression, forced to go through zero, is also shown (slope = 1.10; r^2 = 0.98). Filled circles are data. Dashed line is a perfect match. Solid line is the linear regression.

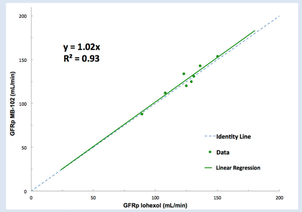

Figure 6: GFR Comparison – Pilot 1B

8 subjects were dosed at 4 µmol/kg. Glomerular Filtration Rate (GFR) from the time-dependent plasma concentration of MB-102 is plotted against the GFR from the time-dependent plasma concentration of iohexol. A linear regression, forced to go through zero, is also shown (slope = 1.02; r^2 = 0.93). Filled circles are data. Dashed line is a perfect match. Solid line is the linear regression.

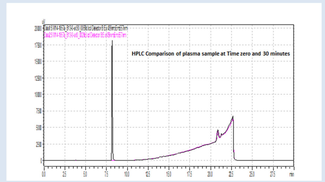

Figure 7: Metabolite Determination of MB-102 in plasma

Plasma chromatograms from the same subject at pre-dose and 30 minutes post-dose are superimposed in order to assess for possible metabolites. Detailed analysis indicates negligible metabolites.

FLUORESCENCE PHARMACOKINETICS

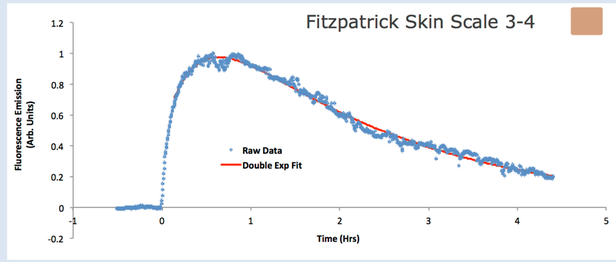

Figure 8: Transdermal fluorescence from MB-102 measured at the sternum in Subject 12 of Pilot 1B.

This data as well as the data for all subjects fit a two-compartment pharmacokinetic model, with the first compartment being the vascular-to-tissue distribution and equilibrium occurring upon administration of each agent, and the second compartment is the elimination phase resulting from renal excretion only. The terminal (second compartment) half-life is approximately 127 minutes. Blue circles are measured values. Red line is a fit to the pharmacokinetic model.

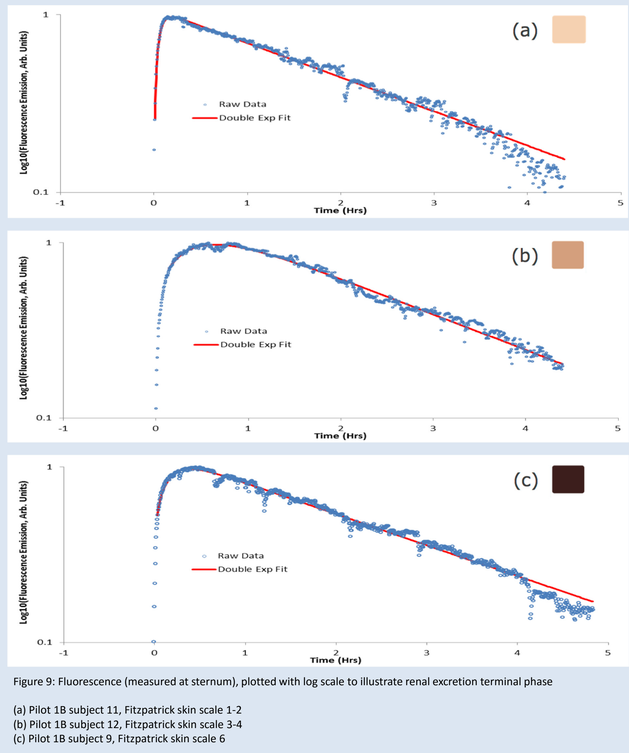

Figure 9: Fluorescence (measured at sternum), plotted with log scale to illustrate renal excretion terminal phase

(a) Pilot 1B subject 11, Fitzpatrick skin scale 1-2
(b) Pilot 1B subject 12, Fitzpatrick skin scale 3-4
(c) Pilot 1B subject 9, Fitzpatrick skin scale 6

The instrument is shown to measure transdermal fluorescence of MB-102 from subjects with a wide range of skin coloration.

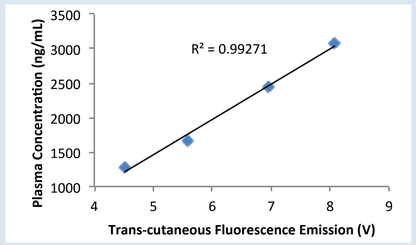

Figure 10: Correlation between plasma pharmacokinetics and fluorescence pharmacokinetics in Pilot 1B, subject 12

Graphing plasma concentration versus fluorescence intensity at the same time points in the renal excretion phase yields an almost perfect correlation. This was true for all 8 subjects that received the 4 mmol/kg dose (data not shown).

RECOVERY OF INJECTED DOSE IN URINE

Table 1: Percent of injected dose (ID) recovered in urine

Pilot Study	# of Subjects	% ID MB-102 Recovered in Urine	% ID Iohexol Recovered in Urine
1A	8	104% +/-3%	86% +/-4%
1B	15	96% +/-8%	98% +/-7%
1 (A and B totaled together)	23	99% +/-7%	94% +/-8%

Collection of all urine was obtained as best as could be done in a non-catheterized setting during the 12-hour post-dosing period of the agents for 23 subjects in the clinical study. The % injected dose of MB-102 recovered in the urine over this 12- hour time interval post-administration matched that of iohexol, which is the agreed upon GFR tracer agent standard. Furthermore, the % injected dose of MB-102 recovered in the urine for these human clinical studies match values found in the animal model previously published (Rajagopalan, R., et al., 2011; Table 2, compound 2d).

CONCLUSION

The results of our first human clinical trial demonstrate the feasibility of noninvasive detection for measurement of GFR employing the fluorescent agent MB-102. In subjects recruited with normal eGFR, we have demonstrated that:

- MB-102 is excreted nearly entirely by the renal system as measured by percent administered dose appearing in the urine, comparing as well or better than the standard iohexol, and hence is a renal tracer agent in humans.
- The measured GFR from MB-102 plasma pharmacokinetics matches the measured GFR from iohexol plasma pharmacokinetics, and hence MB-102 is a GFR tracer agent in humans.
- The plasma pharmacokinetics of MB-102 matches the transdermal fluorescence pharmacokinetics, and hence GFR may now be developed as a noninvasive measurement.

Our next trial will enroll subjects with impaired renal function, and employ a more sensitive detection design suitable for commercial development.

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