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Pre-clinical toxicity evaluation of MB-102, a novel fluorescent tracer agent for real-time measurement of glomerular filtration rate

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ABSTRACT

The fluorescent tracer agent 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine, designated MB-102, has been developed with properties and attributes for use as a direct measure of glomerular filtration rate (GFR). In comparison to known standard exogenous GFR agents in animal models, MB-102 has demonstrated an excellent correlation. A battery of toxicity tests has been completed on this new fluorescent tracer agent, including single dose toxicity studies in rats and dogs to determine overall toxicity and toxicokinetics of the compound. Blood compatibility, mutation assay, chromosomal aberration assay, and several other assays were also completed. Toxicity assessments were based on mortality, clinical signs, body weight, food consumption and anatomical pathology. Doses of up to 200–300 times the estimated human dose were administered. No test-article related effects were noted on body weight, food consumption, ophthalmic observations and no abnormal pathology was seen in either macroscopic or microscopic evaluations of any organs or tissues. All animals survived to scheduled sacrifice. Transient discoloration of skin and urine was noted at the higher dose levels in both species as expected from a highly fluorescent compound and was not considered pathological. Thus initial toxicology studies of this new fluorescent tracer agent MB-102 have resulted in negligible demonstrable pathological test article concerns.

1. Introduction

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).

The current clinical standard is a measurement of serum creatinine and its use in any of the estimated GFR equations (Ferguson and Waikar, 2012; Inker et al., 2012). However this methodology is well-known to be a poor surrogate for a measured GFR as it is rather insensitive (a person can lose up to half their kidney function before an abnormal level of serum creatinine is observed), and is time-delayed (an insult/injury to the kidney would be noted only after 24–48–72 h) (Endre et al., 2010; Star, 1998). In addition, factors not related to renal function affect serum creatinine such as age, hydration, muscle mass, diet, etc. Therefore, the measurement itself is often inaccurate as well.

To overcome the deficiencies of the research GFR tracer agents and the current clinical standard, 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). In rodents, this compound is freely filtered by the kidneys, is not secreted by the renal tubules, nor has demonstrated any significant metabolism *in vivo* (MediBeacon, unpublished results). In dogs, MB-102 has demonstrated similar clearance curves when compared to known exogenous GFR tracer agents such as iohexol and iothalamate, suggesting that MB-102 will provide a similar GFR value and accurate status of overall kidney function in humans. Our noninvasive transdermal measurement methodology as applied to animal models has been

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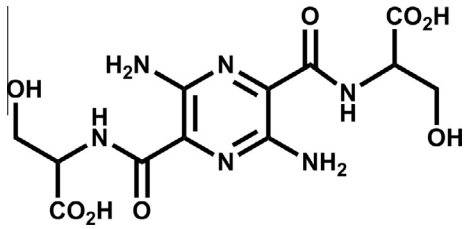

Fig. 1. Structure of 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl} pyrazine.

published elsewhere (Poreddy et al., 2012; Rajagopalan et al., 2011).

The aim of the formal battery of safety and toxicity studies *(in vitro* and *in vivo*) reported herein was to investigate overall toxicity and toxicokinetics of MB-102 in both rodents and dogs necessary to proceed to first-in-human clinical evaluation of this compound. Overall the *in vitro* assays indicated no toxicity relating to CYP-450, cloned human *Ether-à-go-go*-related (hERG) potassium channels, bacterial reverse mutation or chromosomal alteration assays. Additionally, the compound was totally compatible with human blood and plasma samples. *In vivo*, the compound exhibited negligible potential toxicity in a series of CNS, respiratory, and cardiovascular studies in both rodents and dogs. The *in vivo* results suggest that the No Observable Effect Level (NOEL) in rats is greater than 1200 μmol/kg and in dogs is at least 200 μmol/kg.

Thus results of the studies herein confirm that MB-102 is a safe compound and should allow the continuation to a first-in-human clinical trial (pending regulatory clearance). Additional nonclinical studies including biodistribution, drug interference, multi-week toxicity, and several developmental toxicity tests will be completed following a successful first-in-human study. These will be reported upon completion of such following the first-in-human results.

2. Materials and methods

2.1. Fluorescent tracer agent

MB-102 is a fluorescent compound belonging to the general class of compounds known as pyrazines. The chemical structure is shown in Fig. 1. The chemical name is 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine. MB-102 has a molecular weight of 372.3, with light absorption and emission maxima at 445 nm and 560 nm, respectively (Rajagopalan et al., 2011). MB-102 has no structural relationship to other molecules that are known to be carcinogenic or raise other toxicity safety issues.

The safety of pyrazines in general has been demonstrated by their use as medicinal products such as amiloride, a potassium-sparing diuretic on the market for over 30 years. Amiloride hydrochloride (marketed as Midamor) was approved prior to 1982. Natural pyrazines are found in common foods such as beef, bell peppers, cocoa butter, coffee beans, green peas, and potatoes.

2.2. Assays – overview

The *in vitro* and *in vivo* assays are summarized in Table 1.

All animal studies were conducted under the approval of the Institutional Animal Care and Use Committees (IACUC) at the respective Contract Laboratories (Covance Laboratories, Madison, WI and Ricerca, Concord, OH) prior to initiation of the individual *in vivo* studies as described. Male and female rats were Sprague–Dawley (Hsd) and were received from Harlan Laboratories, Indianapolis, IN or Crl:CD(SD) rats obtained from Charles River Laboratories, Portage MI. These animals were housed individually in temperature (23.9 ± 2.2 °C) and humidity (55 ± 4%) controlled rooms, under a 12 h light/dark cycle. The rats were fed Harlan Teklad Certified Global Diet 2016 ad libitum, but fasted prior to study as noted and allowed filtered water for drinking. The beagle dogs were obtained from Harlan Laboratories or Covance Research Laboratories, housed in separate cages in controlled temperature and humidity rooms, and were fed with #2027 certified canine chow unless fasted as noted in study procedures. All of the animal studies were conducted according to the Regulations of Good Laboratory Practice (GLP) for non-clinical laboratory studies issued by the Food and Drug Administration (FDA).

2.3. In vitro assays

2.3.1. Hemolytic potential and blood compatibility in human blood and plasma

Blood (~20 mL) was collected from a fasted human into heparinized tubes. Plasma was harvested from a portion of the collected blood. Whole blood and plasma for hemolytic potential testing and plasma for plasma compatibility testing were collected on the day of testing and held at room temperature until used. Hemolytic Potential Testing Test tubes were set up using test article preparations in vehicle at concentrations of 25, 50, and 100 mM; vehicle; and human whole blood, human plasma, and saponin (1%). Positive control tube included human blood incubated with 1% saponin and negative control samples included individual tubes of human plasma incubated with the three concentrations of test article.

Each mixture was incubated for 40–45 min at approximately 37 °C. After incubation, the tubes were centrifuged, and the supernatant was harvested. The amount of hemoglobin in the supernatant plasma of each tube was measured spectrophotometrically on a Roche chemistry analyzer. The concentration of hemoglobin present in the supernatant plasma of the test article and vehicle mixtures was compared with the respective negative control. Hemolysis was present (recorded as a positive test result) if the concentration of hemoglobin was greater than or equal to

Table 1
Summary of experimental parameters for *in vitro* and *in vivo* studies.

In vitro assays		*In vivo* assays		
Study type	Dose/concentration	Study type	Dose/concentration	#Animals/group
Hemolytic potential and blood compatibility in human blood and plasma	25, 50, and 100 mM	Single dose expanded i.v. bolus toxicity and toxicokinetic study in rats	180, 600, and 1200 μmol/kg	Tox: 10M; 10F TK: 9M; 9F
Bacterial reverse mutation assay	5 mg/plate	Single i.v. dose CNS safety/pharmacology study in rats	0, 180, 600, and 1200 μmol/kg	10M; 10F
Chromosomal aberration assay in cultured human peripheral blood lymphocytes	10 mM and lower	Single i.v. dose respiratory function safety/ pharmacology study in rats	0, 180, 600, and 1200 μmol/kg	4M
CYP-450 enzyme screen	NA	Single dose expanded i.v. bolus toxicity and toxicokinetic study in beagle dogs	60, 200, and 600 μmol/kg	4M; 4F
Effect of MB-102 on cloned hERG potassium channels	10 μM; 300 μM	Single i.v. dose cardiovascular safety/ pharmacology study in beagle dogs	0, 60, 200,and 600 μmol/kg	4F