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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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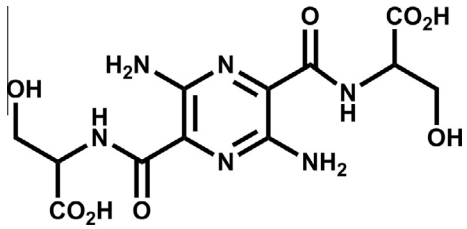

Fig. 1. Structure of 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl} pyrazine.

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

500 mg/dL more than the negative control. Absence of hemolysis, relative to the negative control, was recorded as a negative test result.

Plasma Compatibility Testing using the test article preparations was conducted in vehicle at concentrations of 25, 50, and 100 mM, and vehicle alone. Following the incubation period the contents of each tube were examined macroscopically. Changes in color or clarity, relative to the homologous plasma sample, and the presence of flocculation, precipitation, or coagulation were recorded.

2.3.2. Bacterial reverse mutation assay

The tester strains used in this assay were the Salmonella histidine auxotrophs TA98, TA100, TA1535, and TA1537 (McCann et al., 1975) and the *Escherichia coli* tryptophan auxotroph WP2uvrA (Green and Muriel, 1976). These procedures were used in both the dose range-finding assay and the mutagenicity assays. Each plate was labeled with a code that identified the test article, test phase, tester strain, activation conditions and dose level. The S9 mix and dilutions of the test article were prepared immediately prior to their use. Treatments were performed by adding 100 μL tester strain and 200 μL of test or vehicle control article to 2.5 mL of molten selective top agar (maintained at 45 ± 2 °C). After the required components had been added, the mixture was vortexed and overlaid onto the surface of 25 mL minimal bottom agar in a 15 × 100 mm petri dish. After the overlay solidified, the plates were inverted and incubated for 52 ± 4 h at 37 ± 2 °C. Cultures were treated in the presence of S9 in an identical manner, except using 2.0 mL undiluted molten selective top agar and adding 500 μL S9 mix. Positive controls were administered using a 50 μL dose volume. Plates which were not evaluated immediately following the incubation period were held at >0 to 10 °C until such time that colony counting and bacterial background lawn evaluation could take place.

A test article is considered to have produced a positive response if it induces a dose-dependent increase in revertant frequency that is ⩾2.0-fold vehicle control values for tester strains TA98, TA100, and WP2uvrA, or ⩾3.0-fold vehicle control values for tester strains TA1535 and TA1537 (Green and Muriel, 1976).

2.3.3. Chromosomal aberration assay in cultured human peripheral blood lymphocytes

Human venous blood from healthy, adult donors (nonsmokers without a history of radiotherapy, chemotherapy, or drug usage, and lacking current viral infections) was drawn into sterile, heparinized Vacutainers. Whole blood cultures were initiated in 15 mL centrifuge tubes by adding ~0.6 mL of fresh heparinized blood into a sufficient volume of culture medium so that the final volume was 10 mL in the assay without metabolic activation after the addition of the test article in its chosen vehicle or was 10 mL in the assay with metabolic activation after the addition of the test article in its chosen vehicle and the S9 activation mix. In the chromosomal aberration assays (OECD, 1997), duplicate cultures were used at each test article concentration, for vehicle controls, and for the positive controls.

For the assay without metabolic activation, 2 days after culture initiation, cells were incubated at 37 ± 2 °C with the test article at predetermined concentrations, vehicle control and positive controls for 3 h. For the assay with metabolic activation, 2 days after culture initiation, cultures were incubated at 37 ± 2 °C for 3 h in the presence of the test article at predetermined concentrations, vehicle control and positive controls, and the S9 activation mix. The cultures were then washed with phosphate-buffered saline, re-fed with complete RPMI 1640 medium and incubated for the rest of the culture period up to the time of harvest with 0.1 μg/mL Colcemid present during the last 2 ± 0.5 h of incubation. The cultures were then harvested (~22 h after initiation of treatment). The cultures were centrifuged, the supernatant discarded, and the cells were swollen with 75 mM KCl hypotonic solution. The cultures were then fixed with absolute methanol: glacial acetic acid (3:1, v/v) fixative. Slides were prepared by dropping the harvested cultures on glass slides and air-dried. The slides were stained with 5% Giemsa solution, air-dried, and mounted permanently for the analysis of mitotic index and chromosomal aberrations.

2.3.4. CYP-450 enzyme series

The CYP-450 Enzyme Series was designed to detect activation or inhibition of specific enzyme systems and to assess receptor binding of a number of common physiological and pharmacological receptor types including several CNS targets recommended by the EMEA to evaluate drug dependence potential of MB-102. Included in this lead profile screen were tests against 73 biochemical markers composed of 5 enzyme systems and 68 receptor assays. The methods employed in this study have been adapted from the scientific literature to maximize reliability and reproducibility. Where presented IC_{50} values were determined by a non-linear, least squares regression analysis using MathIQ. Inhibition constants (Ki) values were calculated using the methods of Cheng and Prusoff (Cheng and Prusoff, 1973) using the observed IC_{50} of the test article, the concentration of the radioligand employed in the assay and the historical values for the K_D of the ligand. Significant responses required > 50% inhibition or stimulation for biochemical assays noted in the assays listed.

2.3.5. Effect of MB-102 on cloned hERG potassium channels

HEK293 cells were stably transfected with hERG cDNA. Stable transfectants were selected by coexpression with the G418 resistance gene incorporated into the expression plasmid. Selection pressure was maintained by including G418 in the culture medium. Cells were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (D-MEM/F-12) supplemented with 10% fetal bovine serum, 100 U/mL penicillin G sodium, 100 μg/mL streptomycin sulfate and 500 μg/mL G418. All experiments were performed at near-physiological temperature (33–35 °C). Each cell acted as its own control.

The positive control was applied to two cells ($n = 2$). The performance of the test system was considered acceptable since application of the positive control elicited a response within ±2 standard deviations from the historic average response. Two concentrations were selected to evaluate the concentration–response relationship based on the outcome of the initial concentration range determination. Each concentration was tested in at least three cells ($n \geqslant 3$). Vehicle control solution was applied to three cells ($n = 3$). Duration of application was approximately 3.8 min, which was longer than the longest test article application in the study.

MB-102 at 10 μM was evaluated in five cells ($n = 5$). A mean inhibitory effect on hERG potassium current amplitude of 6.0% was observed. Based on this result, and the established stability of MB-102 in HB-PBS, an additional nominal concentration (300 μM) was selected to evaluate the concentration–response relationship.

One test article concentration was applied to each cell ($n \geqslant 3$). Peak current was measured during the test ramp. A steady state was maintained for at least 20 s before applying test article or positive control. Peak current was measured until a new steady state was achieved.

2.4. In vivo assays

2.4.1. Single dose expanded intravenous bolus toxicity and toxicokinetic study in rats

For the toxicity testing 10 male and 10 female rats per dosing group were used. For the toxicokinetics 9 male and 9 female rats

Table 2
Assignment and dosing of animals for the single dose Central Nervous System pharmacology study.

Dose group	Number of animals (M/F)	Test article	Dose level (μmol/kg)	Dose level (mg/kg)	Dose concentration (mg/mL)
1	10/10	Vehicle	0	0	0
2	10/10	MB-102	180	66.96	6.2
3	10/10	MB-102	600	223.2	20.8
4	10/10	MB-102	1200	446.4	41.5

Table 3
Summary of Functional Observational Battery (FOB) evaluations conducted in the single dose Central Nervous System pharmacology study.

Home-cage observations:	Open-field evaluations:
Posture	Mobility
Involuntary motor movements	Posture
Biting	Involuntary motor movements
Palpebral closure	Gait abnormalities
Vocalizations	Reactivity to environment (Arousal)
	Stereotypical behavior – any repetitive action
	Bizarre behavior
	Number of rears
	Defecation
	Urination
	Vocalizations
Observations made while handling animals:	Reflex determinations:
Ease of removing animal from cage	Approach response
Ease of handling animal	Touch response
Lacrimation	Auditory response
Color of tears	Tail pinch response
Salivation	Eye blink response
Piloerection	Righting reflex
Fur-appearance	Hind limb extensor strength response
Palpebral closure	Pupillary size
Exophthalmus	
Respiration	

were used per each dosing group evaluated. The test article was supplied as a sterile solution at the concentration (111.66 mM) used for dosing the high-dose group. Prepared doses were stored at room temperature and protected from light in amber glass vials wrapped in foil. Doses were administered by bolus intravenous injection in the tail vein as a single dose on Day 1 of the dosing phase at a dose volume of 10.75 mL/kg. Actual dose volumes were based on the body weight from Day 1 of the dosing phase.

All animals were checked twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. Cage side observations were done for all toxicity animals immediately postdose and approximately 5, 15, 30, 45, and 60 min postdose and for selected toxicity animals approximately 120 min postdose. On nondosing days (except for days of detailed observations), daily cage side observations were made for each toxicity animal. Detailed observations were conducted for all animals once during the predose phase and for toxicity animals before dosing on Day 1 and weekly thereafter and on the day of scheduled sacrifice.

2.4.2. Single dose Central Nervous System (CNS) safety pharmacology study in rats

A total of 80 animals (10/sex/group) were assigned to groups and dosed as shown in Table 2. The animals were not fasted overnight prior to dosing. The animals were evaluated for activity measurements, and a functional observational battery test (FOB) was performed including cage observations, handling observations, open-field observations and handling/specific testing of the animals as described in Table 3.

The test article was administered once, as a slow bolus intravenous tail vein injection, at a dose volume of 10.75 mL/kg body weight. Control animals received the vehicle only at the same dose volume. The actual volume/weight to be administered to each animal was calculated and adjusted based on the Day 1 body weight of each animal.

Animals were observed for viability at least once in the morning and once in the afternoon, at least 4 h apart, throughout the study. The neurobehavioral assessment included a Functional Observational Battery (FOB) and a motor activity test. The FOB and motor activity test were performed on all animals once predose and at three time points postdose (between 1 min and 45 min, between 3 and 4 h, and between 23.5 and 24.5 h). The anticipated T_{max} is 5 min and the $T_{1/2}$ is less than 0.5 h postdose based on previously conducted clearance studies in this species. Animals were randomized prior to dose administration for the purpose of conducting blinded FOB testing throughout the study.

2.4.3. Single dose respiratory safety pharmacology study in rats

A total of 16 male rats were assigned to groups as shown in Table 4. The test article was administered once, as a slow bolus intravenous tail vein injection at a dose volume of 10.75 mL/kg body weight. Control animals received the vehicle at the same dose volume.

The animals were placed in a whole body plethysmograph for a minimum of 1 h prior to dose administration to collect baseline respiratory data. The animals were removed from the chamber briefly and the dose was administered intravenously as a bolus injection via the tail vein. After dose administration, animals were placed back into the plethysmograph chamber, and respiratory data was collected for 2 h on the day of dosing and for 1 h at approximately the 24-h postdose time point. Body weights were recorded on the day of dosing, for dose calculations. Dosing was staggered over 2–4 days by dosing an equal number of animals from each group (1 or 2 per group per day). The following parameters were recorded and reported: respiratory rate

Table 4
Assignment and dosing of male rats in the single dose respiratory safety study.

Dose group	Number of male rats	Test article	Dose level (μmol/kg)	Dose level (mg/kg)	Dose concentration (mg/mL)
1	4	Vehicle	0	0	0
2	4	MB-102	180	66.96	6.2
3	4	MB-102	600	223.2	20.8
4	4	MB-102	1200	446.4	41.5

(breath/min, tidal volume (TV, mL/breath), and minute volume (MV, mL/min).

Four 15-min predose intervals were collected and the last two intervals were averaged together for a single baseline mean value per rat. Postdose data were summarized as means of 5-min intervals for the first 30 min and of 15-min intervals for 30-min to 2-h time period.

2.4.4. Single dose expanded intravenous bolus toxicity and toxicokinetic study in beagle dogs

A total of 16 male and 16 female dogs were used in this study. The animals were dosed as indicated in Table 5, with each treatment group consisting of 4 male and 4 female dogs.

Animals were checked twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. Daily cage side observations were performed during the predose and dosing phases, except on the days that detailed observations were conducted. Detailed observations were performed twice during the predose phase; before dosing on Day 1 and weekly thereafter; and on the day of scheduled sacrifice (only those animals sacrificed that day). Abnormal findings or an indication of normal was recorded.

Detailed observations were also made for each animal immediately postdose and approximately 5, 15, 30, 45, and 60 min postdose and detailed observations continued (for those animals that previously had abnormal skin color or other abnormal observations potentially attributed to the test article) at approximately 2 and 3 h postdose. Abnormal findings or an indication of normal was recorded. Unscheduled observations were recorded. Body weights were recorded twice during the predose phase; prior to dosing on the first day of the dosing phase; and on Days 3, 8, and 15 of the dosing phase.

On Day 15 of the dosing phase, all surviving animals were weighed, anesthetized with sodium pentobarbital, exsanguinated, and necropsied. The necropsy included an examination of the external features of the carcass, external body orifices, the abdominal, thoracic, and cranial cavities, organs, and tissues.

2.4.5. Single dose cardiovascular safety pharmacology study in dogs

A total of 4 female beagle dogs were used in this study. Each dog randomly received Vehicle (0 μmol/kg), Low (60 μmol/kg), Middle (200 μmol/kg) and High (600 μmol/kg) doses of the test article in a balanced Latin-Square design as shown in Table 6, Dosing schedule and Table 7, Doses of MB-102.

Doses were administered as a slow bolus injection (<5 min) in the cephalic vein and a 3-day wash-out duration occurred between sequential dose administrations. The animals were fasted overnight prior to each dose administration, and fed approximately 2 h postdose. The dose levels and the intervals monitored for the study were selected based on available toxicity and toxicokinetic data in this species.

The test article or vehicle was administered once per day with a 3-day washout between doses. All animals were observed once each morning and afternoon throughout the study for morbidity and mortality. On the days of dose administration, physical observations were conducted at approximately 2 and 3 h post-dose (at the time of feeding and removal of food) and all room entry/exit times recorded. On non-dosing days, physical observations were conducted once daily. The dogs for this study were previously surgically implanted with Data Sciences International (DSI, St. Paul, MN) transmitters (TL11M2-D70-PCT). Transmitters were used to record ECG, arterial blood pressures, and body temperature. Animals were unrestrained within their home cages during collection of the selected cardiovascular parameters. Arterial blood pressure, ECG, and body temperature were recorded by telemetry for at least 12 h prior to the first dosing event to verify function of the radiotelemetry unit. On dosing days, data were collected from a minimum 30-min predose period and used to generate a single baseline mean value for each parameter. Data were collected continuously for a minimum of 24 h postdose and during this time data were recorded as mean values of 60-s time bins. Further data summarization was generated as 5-min means for the period 0–30 min postdose, and as 15-min means for the period 30 min to 24 h postdose. Times of any entry into the room during radiotelemetry monitoring were documented.

The following parameters were analyzed: systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP), heart rate (HR) and body temperature (Tb). ECG parameters evaluated included: PR Interval, QRS Interval, QT Interval, QTcV (Van de Water's corrected QT) RR interval. One-minute tracings of the ECGs were obtained at predose, 15 min, 30 min, 1 h, 4 h,

Table 5
Dosing of dogs for the single dose intravenous bolus toxicity and toxicokinetic study.

Group	Number of dogs (male/female)	Dose level (μmol/kg)	Dose concentration (mM)
1 (control)	4/4	0	0
2 (low)	4/4	60	11.2
3 (middle)	4/4	200	37.2
4 (high)	4/4	600	111.6

Table 6
Dosing schedule for the single dose cardiovascular safety study in female dogs.

Animal ID	Dose event 1	Dose event 2	Dose event 3	Dose event 4
1	Vehicle	Low	High	Middle
2	Low	Middle	Vehicle	High
3	Middle	High	Low	Vehicle
4	High	Vehicle	Middle	Low

Table 7
Doses of MB-102 administered during the single dose cardiovascular safety study in female dogs.

Group	Dose volume (mL)	Dose level (mg/kg)	Dose concentration (mg/mL)	Dose level (μmol/kg)
Vehicle	5.38	0	0	0
Low	5.38	22.3	4.14	60
Middle	5.38	74.4	13.83	200
High	5.38	223.4	41.52	600

Table 8
Hemolytic potential and blood compatibility test results.

Human blood plus	[HGB] (mg/dL)	Result[a]
Hemolytic potential		
25 mM MB-102	14	–
50 mM MB-102	10	–
100 mM MB-102	5	–
Vehicle	5	–
1% saponin	5655	+
Human plasma plus	**[HGB] (mg/dL)**	**Result[b]**
Blood compatibility		
25 mM MB-102	19	–
50 mM MB-102	4	–
100 mM MB-102	1	–

Note: Abbreviations used: HGB, hemoglobin.
[a] (+) Result = >500 mg/dL [HGB].
[b] (+) Result = changes in color or clarity; presence of flocculation, precipitation, or coagulation.

and at 24 h. ECG traces were evaluated and reported by a board-certified veterinary cardiologist.

3. Results

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

The results for human blood mixed with the 25, 50, or 100 mM test article concentrations, or vehicle, or 1% Saponin are listed in Table 8. The levels of hemoglobin in all the tubes were markedly lower (<14 mg/dL) than the positive control tube containing 1% Saponin (5655 mg/dL). Negative control samples prepared with equal volumes of human plasma mixed with the 25, 50, and 100 mM MB-102 concentrations, to account for the colored nature of MB-102, also resulted in markedly low levels of hemoglobin (<19 mg/dL). No changes were observed macroscopically compared with homologous plasma or with vehicle. Results also showed that no hemolysis occurred when MB-102 was mixed with human whole blood. Additionally, human plasma was treated with MB-102 at the concentrations noted and evaluated for changes in color or clarity, relative to the homologous plasma sample, and the presence of flocculation, precipitation, or coagulation. The accompanying data demonstrate that MB-102 is compatible with human whole blood and plasma.

3.2. Bacterial reverse mutation assay

MB-102 was evaluated in the dose range-finding assay in tester strains TA100 and WP2uvrA. Ten doses of test article, ranging from 6.67 to 5000 μg/plate, were evaluated with and without S9. No cytotoxicity was observed with either tester strain in the presence or absence of S9 as evidenced by no dose-related decreases in revertant frequency and normal bacterial background lawns.

Based upon the results of the dose range-finding assay, MB-102 was evaluated in the initial mutagenicity assay, in all five tester strains, at doses ranging from 313 to 5000 μg/plate in the presence and absence of S9 mix. All doses of the test article, as well as the concurrent positive and vehicle controls, were evaluated in triplicate plates. No positive increases in the mean number of revertants/plate were observed with any of the tester strains in the presence or absence of S9 mix. The data shown in Table 9A are with S9 present, and the data shown in Table 9B are in the absence of S9.

There was no dose dependency seen in any of the tester strains. The tester strain:vehicle (T:V) ratio was not greater than 2 for the tester strains TA98, TA100, and WP2uvrA. The T:V ratio was not greater than 3 for the tester strains TA1535 and TA1537. These results indicate that MB-102 was negative in the Bacterial Reverse Mutation Assay.

3.3. Chromosomal aberration assay in cultured human peripheral blood lymphocytes

The initial chromosomal aberration assay was conducted by testing 15 concentrations of MB-102 ranging from 0.0678 to 10.0 mM without and with metabolic activation. All cultures were harvested ~22 h from the initiation of treatment. Chromosomal aberrations were analyzed from the cultures treated with 4.90, 7.00, and 10.0 mM MB-102 without and with metabolic activation. The highest dose selected for analysis, 10.0 mM, is the high dose recommended for this assay by the OECD Testing Guidelines (OECD, 1997). No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed without (data not shown) or with metabolic activation (Table 10). The test article, MB-102, was negative for inducing chromosomal aberrations in cultured human lymphocytes without and with metabolic activation.

Table 9A
Bacterial reverse mutation assay results (with S9).

Strain	Agent	Dose (μg/plate)	Mean revertants/plate	T:V
TA98	MB-102	5000	21.0 ± 2.0	1.1
		2500	17.3 ± 1.5	0.9
		1250	21.7 ± 11.0	1.1
		625	26.3 ± 2.3	1.4
		313	18.7 ± 2.9	1.0
	PBS	–	19.3 ± 5.1	–
TA100	MB-102	5000	86.0 ± 4.6	1.0
		2500	80.0 ± 7.0	0.9
		1250	80.0 ± 4.6	0.9
		625	78.0 ± 13.1	0.9
		313	82.7 ± 5.8	1.0
	PBS	–	85.7 ± 5.1	–
TA1535	MB-102	5000	11.0 ± 4.6	1.5
		2500	8.0 ± 2.6	1.1
		1250	10.3 ± 2.1	1.4
		625	7.3 ± 2.5	1.0
		313	9.0 ± 6.1	1.2
	PBS	–	7.3 ± 2.5	–
TA1537	MB-102	5000	5.3 ± 0.6	1.0
		2500	4.3 ± 0.6	0.8
		1250	5.3 ± 1.5	1.0
		625	5.7 ± 2.1	1.1
		313	6.3 ± 1.5	1.2
	PBS	–	5.3 ± 1.5	–
WP2uvrA	MB-102	5000	13.7 ± 1.6	0.8
		2500	11.3 ± 1.5	0.7
		1250	18.0 ± 3.0	1.1
		625	19.3 ± 3.5	1.2
		313	20.3 ± 4.2	1.2
	PBS	–	16.3 ± 2.3	–
TA98	BP	2.5	263.7 ± 21.5	13.6
TA100	2AA	2.5	1121.3 ± 367.2	13.1
TA1535	2AA	2.5	151.7 ± 26.0	20.7
TA1537	2AA	2.5	54.7 ± 13.1	10.3
WP2uvrA	2AA	25.0	404.3 ± 52.8	24.8

Note: Results presented as mean ± standard deviation. *Abbreviations used:* T:V, tester strain to vehicle ratio; PBS, phosphate buffered saline; BP, Benzo(a)pyrene; 2AA, 2-aminoanthracene.

3.4. CYP-450 enzyme series

There were no significant findings (>50% stimulation or inhibition) for any of the enzyme system or receptors assayed (data not shown). Included in this panel of assays were α (1A, 2A, 1B) and β (B1, B2) adrenergic receptors, dopamine (D_1, D_{25}, D3, D42), bradykinin (B1, B2), histamine (H_1, H_2, H_3), neuropeptide Y (Y1, Y2), and opiate (δ, κ, μ) receptors. The CYP-450 enzyme systems assayed included 1A2, 2C19, 2C9, 2D6 and 3A4. These results indicate that MB-102 does not cause significant activation or inhibition of the enzyme systems tested or bind to the wide cross section of receptors evaluated.

3.5. Effects on cloned hERG channels

The *in vitro* effects of MB-102 on ionic currents in voltage-clamped human embryonic kidney cells (HEK293) that stably express the human *Ether-à-go-go*-related gene (hERG) were determined. Two concentrations of MB-102 (10 and 300 μM) were tested at near-physiological temperature. Each recording ended with a final application of a supramaximal concentration of the reference substance (E-4031, 500 nM) to assess the contribution of endogenous currents. The remaining uninhibited current was subtracted off-line digitally from the data to determine the potency of the test

Table 9B
Bacterial reverse mutation assay results (without S9).

Strain	Agent	Dose (μg/plate)	Mean Revertants/Plate	T:V
TA98	MB-102	5000	14.3 ± 3.2	0.8
		2500	13.7 ± 2.1	0.8
		1250	12.7 ± 2.9	0.7
		625	16.3 ± 4.2	1.0
		313	15.7 ± 2.3	0.9
PBS		–	17.0 ± 0.0	–
TA100	MB-102	5000	74.3 ± 7.5	0.9
		2500	73.7 ± 9.9	0.9
		1250	74.7 ± 15.0	0.9
		625	77.7 ± 2.3	0.9
		313	84.7 ± 11.2	1.0
PBS		–	83.3 ± 18.2	–
TA1535	MB-102	5000	7.7 ± 2.1	1.0
		2500	8.3 ± 1.5	1.1
		1250	10.3 ± 2.5	1.4
		625	8.7 ± 3.2	1.2
		313	11.3 ± 1.5	1.5
PBS		–	7.3 ± 2.3	–
TA1537	MB-102	5000	5.0 ± 2.0	1.2
		2500	3.3 ± 1.2	0.8
		1250	2.7 ± 1.5	0.6
		625	8.0 ± 2.6	1.8
		313	5.7 ± 1.9	1.3
PBS		–	4.3 ± 0.6	–
WP2uvrA	MB-102	5000	16.0 ± 3.6	0.9
		2500	20.0 ± 6.2	1.1
		1250	16.3 ± 1.2	0.9
		625	13.0 ± 2.0	0.7
		313	18.0 ± 1.7	1.0
	PBS	–	18.3 ± 10.1	–
TA98	2NF	2.5	197.3 ± 33.2	11.6
TA100	SA	2.5	782.3 ± 153.2	9.4
TA1535	SA	2.5	572.0 ± 14.2	78.0
TA1537	ICR	2.5	191.3 ± 43.1	44.2
WP2uvrA	4NQO	25.0	1165 ± 10.5	9.0

Note: Results presented as mean ± standard deviation. *Abbreviations used:* T:V, tester strain to vehicle ratio; PBS, phosphate buffered saline; 2NF, 2-nitrofluorene; SA, sodium azide; ICR, acridine mutagen ICR-191; 4NQO, 4-nitroquinoline *N*-oxide.

substance for hERG inhibition. The results are shown in Table 11. The IC_{50} for the inhibitory effect of MB-102 on hERG potassium current was not calculated but was estimated to be greater than 300 μM.

In Fig. 2, the upper panel shows superimposed records of hERG potassium currents obtained in a single cell during application of vehicle control, test article and reference substance. hERG potassium currents were evoked by the voltage protocol shown in the lower panel. Based on the lack of significant change in hERG channel potentials for the three concentrations of MB-102 evaluated, the data demonstrate that no effect on hERG channel inhibition was observed between 0-300 μM concentrations of MB-102.

3.6. Single dose expanded intravenous bolus toxicity and toxicokinetic study in rats

All animals survived to their scheduled sacrifice. MB-102-related clinical signs were observed in treated animals at all dose levels tested. The most common clinical signs included yellow discoloration of the skin at various anatomic locations including the tail, paws, ears and nose, and/or discolored urine (yellow or orange). These observations were attributed to the colored nature of the test article and were not deemed adverse. Yellow discoloration of the skin was noticed at all dose levels, although it was more widespread and appeared more rapidly in animals given 600 or 1200 μmol/kg. Discolored urine (yellow or orange) was observed primarily in animals give >600 μmol/kg. No other abnormal clinical signs were noted after Day 3 of the dosing phase. No test article-related effects on body weight or body weight gain were observed during the dosing phase. No test article-related alteration in food consumption was noted. No abnormal ophthalmic observations were noted.

The mean concentration–time profiles (data not shown) for males and females show that exposure to MB-102 increased with the increase in MB-102 dose level from 180 to 1200 μmol/kg. After intravenous bolus administration, MB-102 concentrations readily declined generally in a mono-exponential manner and with concentrations below the lower limit of quantitation by 24 h. The $t_{1/2}$ values ranged from 0.392 to 0.417 h. Values for $t_{1/2}$ were generally dose independent. Values for clearance rate ranged from 0.402 to 0.485 L/h/kg and appeared to be dose independent.

Test article-related macroscopic findings were limited to yellow discoloration of intravenous sites in a limited number of animals observed. The yellow discoloration of the intravenous sites was attributed to the yellow color of the test article and was not deemed to be toxicity related. The remaining macroscopic findings in interim sacrifice animals were incidental or normal background findings commonly reported in animals of this species and age.

The microscopic findings in interim sacrifice animals were incidental or normal background findings commonly reported in animals of this species and age. All interim sacrifice animals survived to their scheduled sacrifice. Significant absolute and relative organ weight changes in interim sacrifice animals were few in number, random in nature, not dose-responsive, and not test article-related.

All terminal sacrifice animals survived to their scheduled sacrifice. Significant relative organ weight changes in terminal sacrifice animals were few in number, random in nature, not dose-responsive, and not test article-related. Microscopic observations upon sacrifice were made on many tissues including bone, spinal cord, nerve, pituitary, adrenal, aorta, trachea, thyroid, parathyroid, lung, liver, gallbladder, kidney, bladder, esophagus, stomach, duodenum, colon, ileum, jejunum, cecum, rectum, pancreas, spleen, thymus, muscle, tongue, mammary, prostate, testis, epididymis, ovary, uterus, vagina, eye, heart, and brain. The macroscopic and microscopic findings in terminal sacrifice animals were incidental or normal background findings commonly reported in animals of this species and age.

Values for volume at steady state (V_{ss}) ranged from 0.192 to 0.205 L/kg and were also dose independent. Values for V_{ss} were less than the total body water of a 0.25 kg rat but greater than the blood volume, indicating that MB-102 was moderately distributed after intravenous bolus administration of MB-102.

Table 10
Chromosomal aberration assay results (with metabolic activation).

Treatment	Concentration (mM)	% Mitotic index culture A	% Mitotic index culture B	Mean mitotic index	% Mitotic reduction
Vehicle	–	10.6	9.4	10.0	0
MB-102	3.43	10.4	10.7	10.6	0
MB-102	4.90	10.8	11.6	11.2	0
MB-102	7.00	10.0	9.6	9.8	2
MB-102	10.0	9.7	9.5	9.6	4

Table 11
hERG channel assay results.

Compound	[Concentration]	Mean % inhibition	SEM	*N*
MB-102	0 μM	1.9	0.8	3
MB-102	10 μM	6.0	3.1	5
MB-102	300 μM	2.9	0.7	3
Terfenadine	60 nM	85.2	3.6	2

Note: Abbreviations used: SEM, standard error of the mean. Terfenadine employed as a positive control.

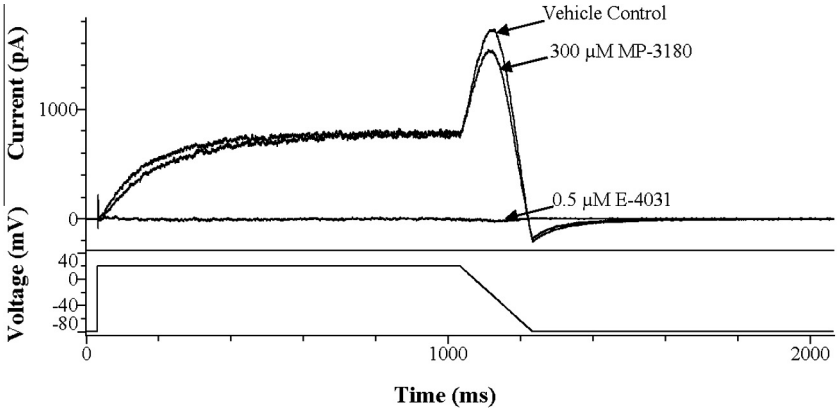

Fig. 2. hERG potassium current traces.

Table 12A
Hematological parameters from male rats (n = 10/group) 3 days post dosing of MB-102.

Parameter	Dose (μmol/kg)			
	0 μmol/kg	180 μmol/kg	600 μmol/kg	1200 μmol/kg
RBC (e6/μL)	7.67 ± 0.298	7.76 ± 0.367	7.60 ± 0.328	7.78 ± 0.275
WBC (e5/μL)	6.82 ± 0.888	7.73 ± 1.004	7.21 ± 0.844	7.78 ± 1.590
Baso (e3/μL)	0.02 ± 0.009	0.02 ± 0.009	0.02 ± 0.008	0.02 ± 0.009
Mono (e3/μL)	0.10 ± 0.027	0.11 ± 0.051	0.11 ± 0.035	0.13 ± 0.063
Luc (e3/μL)	0.05 ± 0.017	0.05 ± 0.002	0.05 ± 0.009	0.07 ± 0.020
Eos (e3/μL)	0.07 ± 0.057	0.04 ± 0.021	0.04 ± 0.025	0.04 ± 0.014
Neut (e3/μL)	0.61 ± 0.415	0.70 ± 0.219	0.66 ± 0.167	0.82 ± 0.329
Lym (e3/uL)	5.97 ± 0.979	6.50 ± 0.849	6.34 ± 0.742	6.70 ± 1.485
Plt (e3/μL)	1508 ± 204.8	1441 ± 253.5	1525 ± 190.1	1505 ± 172.2
HGB (g/dL)	14.5 ± 0.34	14.4 ± 0.58	14.3 ± 0.49	14.2 ± 0.51
Hct (%)	47.3 ± 1.09	47.1 ± 1.78	46.4 ± 1.98	46.6 ± 1.54
[a]PT (s)	18.6 ± 0.36	18.9 ± 0.372	18.9 ± 0.59	19.0 ± 0.44
[a]APTT (s)	20.8 ± 2.30	20.2 ± 1.88	21.3 ± 0.91	21.1 ± 1.73

Note: Results presented as mean ± standard deviation. *Abbreviations used:* RBC, red blood cell count; WBC, white blood cell count; Baso, basophil granulocytes; Mono, monocytes; Luc, large unstained cells; Eos, eosinophil granulocytes; Neut, neutrophil granulocytes; Lym, lymphocytes; Plt, platelets; HGB, hemoglobin; Hct, hematocrit; PT(s), prothrombin time; APTT(s), activated partial thromboplastin time.

[a] PT and APTT performed on Day 15 post-dosing, n = 5/group.

No sex differences (>2-fold) were observed in the MB-102 maximum plasma concentration (C_{max}) and area under the plasma concentration–time curve (AUC_{0-24}) values. The increases in C_{max} and AUC_{0-24} for males and females were, in general, dose proportional. Few statistically significant or otherwise notable differences for hematological parameters (Table 12A and B) and clinical chemistry test results were observed between control and treated animals (Table 13A and B). All of the differences were consistent with normal variation and considered incidental. All of the differences were characterized by one or more of the following: very small magnitude, no relationship to dose, inconsistency between sexes, and absence of correlative findings.

3.7. Single dose CNS safety pharmacology study in rats

A neurobehavioral assessment using a Functional Observational Battery (FOB) and a motor activity test were performed on all animals pretest and postdose at 1–45 min, 3–4 h, and 23.5–24.5 h. There were no test article-related changes in FOB or motor activity parameters. Table 14 summarizes the 1–45 min data. Remaining data were unremarkable and were not tabulated.

3.8. Single dose respiratory safety pharmacology study in rats

Respiratory rate was significantly higher (p = 0.05; per Bonferroni method) at 30 min postdose for rats receiving 180 or

Table 12B
Hematological parameters from female rats (n = 10/group) 3 days post dosing of MB-102.

Parameter	Dose (μmol/kg)			
	0 μmol/kg ± SD	180 μmol/kg ± SD	600 μmol/kg ± SD	1200 μmol/kg ± SD
RBC (e6/μL)	7.56 ± 0.474	7.61 ± 0.349	7.95 ± 0.321	7.87 ± 0.348
WBC (e5/μL)	5.33 ± 0.894	5.51 ± 1.148	5.68 ± 1.193	5.87 ± 0.765
Baso (e3/μL)	0.01 ± 0.000	0.01 ± 0.006	0.01 ± 0.006	0.02 ± 0.009
Mono (e3/μL)	0.07 ± 0.024	0.09 ± 0.004	0.08 ± 0.034	0.09 ± 0.018
Luc (e3/μL)	0.04 ± 0.013	0.04 ± 0.019	0.04 ± 0.012	0.04 ± 0.011
Eos (e3/μL)	0.06 ± 0.011	0.07 ± 0.024	0.08 ± 0.034	0.08 ± 0.042
Neut (e3/μL)	0.64 ± 0.188	0.63 ± 0.246	0.66 ± 0.315	0.69 ± 0.133
Lym (e3/uL)	4.51 ± 0.869	4.67 ± 0.797	4.81 ± 0.859	4.95 ± 0.671
Plt (e3/μL)	1543 ± 150.5	1251 ± 88.3	1234 ± 354.9	1276 ± 74.6
HGB (g/dL)	14.1 ± 0.90	14.63 ± 0.79	14.7 ± 0.43	14.3 ± 1.02
Hct (%)	44.6 ± 2.69	44.9 ± 2.50	46.5 ± 0.91	45.8 ± 2.05
[a]PT (s)	19.3 ± 0.40	20.2 ± 2.59	19.1 ± 0.58	19.1 ± 0.81
[a]APTT (s)	20.8 ± 1.44	21.0 ± 3.55	19.6 ± 0.48	19.3 ± 1.71

Note: Results presented as mean ± standard deviation. *Abbreviations used:* RBC, red blood cell count; WBC, white blood cell count; Baso, basophil granulocytes; Mono, monocytes; Luc, large unstained cells; Eos, eosinophil granulocytes; Neut, neutrophil granulocytes; Lym, lymphocytes; Plt, platelets; HGB, hemoglobin; Hct, hematocrit; PT(s), prothrombin time; APTT(s), activated partial thromboplastin time.

[a] PT and APTT performed on Day 15 post-dosing, n = 5/group.

Table 13A
Clinical chemistry parameters from male rats (n = 10/group) 3 days post dosing of MB-102.

Parameter	Dose (μmol/kg)			
	0 μmol/kg	180 μmol/kg	600 μmol/kg	1200 μmol/kg
Glu (mg/dL)	96 ± 8.9	98 ± 7.8	100 ± 7.4	97 ± 5.0
Alb (g/L)	4.4 ± 0.09	4.4 ± 0.13	4.3 ± 0.08	4.4 ± 0.13
Chol (mg/dL)	124 ± 19.1	116 ± 14.1	128 ± 19.3	126 ± 12.1
Crea (mg/dL)	0.6 ± 0.05	0.6 ± 0.05	0.6 ± 0.0	0.6 ± 0.04
TBIL (μmol/L)	0.1 ± 0.03	0.01 ± 0.00	0.1 ± 0.00	0.1 ± 0.00
Phos (mg/dL)	9.5 ± 0.45	9.5 ± 0.32	9.5 ± 0.46	9.4 ± 0.41
TP (g/dL)	6.2 ± 0.19	6.3 ± 0.21	6.0 ± 0.18	6.2 ± 0.17
UN (mg/dL)	18.0 ± 3.5	20.0 ± 2.5	20.0 ± 1.5	19.0 ± 1.7
GLOB (g/L)	1.8 ± 0.14	1.9 ± 0.13	1.8 ± 0.13	1.8 ± 0.13
AGR	2.4 ± 0.21	2.3 ± 0.15	2.4 ± 0.15	2.4 ± 0.21
ALP (U/L)	156 ± 21	142 ± 12.8	149 ± 19.9	153 ± 18.7
AST (U/L)	122 ± 14.9	115 ± 16.4	110 ± 13.6	111 ± 15.4
ALT (U/L)	43 ± 3.0	42 ± 3.1	44 ± 4.8	41 ± 4.0
Na^+ (mmol/L)	144 ± 1.2	145 ± 1.3	144 ± 1.2	145 ± 1.2
K^+ (mmol/L)	6.1 ± 0.21	6.1 ± 0.39	6.0 ± 0.37	6.0 ± 0.36
Ca^{++} (mmol/L)	11.0 ± 0.18	11.1 ± 0.25	11.0 ± 0.19	11.1 ± 0.16
Cl^- (mmol/L)	103 ± 1.6	103 ± 0.9	103 ± 1.1	102 ± 0.8

Note: Results presented as mean ± standard deviation. *Abbreviations used:* Glu, glucose; Alb, albumin; Chol, cholesterol; Crea, creatinine; TBIL, total bilirubin; Phos, phosphate; TP, total protein; UN, blood urea nitrogen; GLOB, globulins; AGR, albumin-globulin ratio; ALP, alkaline phosphatase; AST, aspartate transaminase; ALT, alanine transaminase; Na^+, sodium; K^+, potassium; Ca^{++}, calcium; Cl^-, chloride.

600 μmol/kg of MB-102. Tidal volume was significantly lower (p = 0.05; per Bonferroni method) than control at 10 min postdose (1200 μmol/kg), and at 23.75 h postdose (180, 600, 1200 μmol/kg). These findings were not considered test article-related as there were no clear correlations to dose level or trends following dose administration. Minute volume had no significant changes between dose groups.

There were no test article-related changes in the respiratory parameters measured. The NOEL (No-Observable Effect Level) on respiratory parameters in the rat was greater than 1200 μmol/kg, the high dose of this study, when given as a single intravenous bolus injection.

Table 13B
Clinical chemistry parameters from female rats 3 days post dosing of MB-102.

Parameter	Dose (μmol/kg)			
	0 μmol/kg	180 μmol/kg	600 μmol/kg	1200 μmol/kg
Glu (mg/dL)	109 ± 10.8	107 ± 8.6	104 ± 9.3	108 ± 8.3
Alb (g/L)	4.6 ± 0.14	4.6 ± 0.22	4.6 ± 0.15	4.6 ± 0.14
Chol (mg/dL)	108 ± 12.2	107 ± 11.4	100 ± 14.1	111 ± 15.4
Crea (mg/dL)	0.7 ± 0.06	0.7 ± 0.03	0.7 ± 0.04	0.7 ± 0.03
TBIL (μmol/L)	0.1 ± 0.00	0.01 ± 0.00	0.1 ± 0.00	0.1 ± 0.00
Phos (mg/dL)	9.5 ± 0.45	9.5 ± 0.32	9.5 ± 0.46	9.4 ± 0.41
TP (g/dL)	6.4 ± 0.29	6.5 ± 0.32	6.5 ± 0.25	6.5 ± 0.22
UN (mg/dL)	19 ± 1.6	20 ± 2.8	20 ± 2.8	20 ± 1.5
GLOB (g/L)	1.8 ± 0.21	1.9 ± 0.15	1.9 ± 0.14	1.9 ± 0.10
AGR	2.6 ± 0.3	2.5 ± 0.17	2.4 ± 0.15	2.4 ± 0.15
ALP (U/L)	83 ± 8.2	84 ± 6.0	84 ± 6.2	83 ± 9.9
AST (U/L)	96 ± 10.7	105 ± 11.5	[a]113 ± 17.4	108 ± 10.6
ALT (U/L)	33 ± 3.3	[a]38 ± 3.1	[a]39 ± 4.2	34 ± 2.4
Na^+ (mmol/L)	144 ± 1.1	144 ± 1.4	144 ± 1.7	144 ± 1.3
K^+ (mmol/L)	5.5 ± 0.54	5.4 ± 0.42	5.7 ± 0.32	5.7 ± 0.40
Ca^{++} (mmol/L)	11.2 ± 0.26	11.1 ± 0.24	11.2 ± 0.20	11.2 ± 0.21
Cl^- (mmol/L)	103 ± 1.6	103 ± 0.9	102 ± 2.3	103 ± 1.3

Note: Results presented as mean ± standard deviation. *Abbreviations used:* Glu, glucose; Alb, albumin; Chol, cholesterol; Crea, creatinine; TBIL, total bilirubin; Phos, phosphate; TP, total protein; UN, blood urea nitrogen; GLOB, globulins; AGR, albumin-globulin ratio; ALP, alkaline phosphatase; AST, aspartate transaminase; ALT, alanine transaminase; Na^+, sodium; K^+, potassium; Ca^{++}, calcium; Cl^-, chloride.
[a] Significantly different ($p \leqslant 0.05$) from baseline values per one-way analysis of variance (ANOVA).

3.9. Single Dose expanded intravenous bolus toxicity and toxicokinetic study in beagle dogs

All animals survived to their scheduled sacrifice. MB-102-related clinical signs included yellow discoloration of various anatomic locations and discolored urine (yellow or orange). These were attributed to the colored nature of the test article and were not considered adverse. Yellow discoloration of various anatomic locations was primarily seen on Day 1 of the dosing phase in animals given 200 or 600 μmol/kg. These locations included eyes, gums, ears, legs, and abdomen. At 2 h postdose, yellow discoloration appeared to fade from certain body parts and was localized to one or more sites. By 3 h postdose, the color of all body parts was normal. Discolored urine (yellow or orange) was observed in all three MB-102-treated groups on Day 1 of the dosing phase.

No MB-102-related effects on body weight or body weight gain were observed during the dosing phase. No effects were noted on food consumption. Significant relative organ weight changes in terminal sacrifice animals were few in number, random in nature, not dose-responsive, and not test article-related. The macroscopic and microscopic findings in both the interim and terminal sacrifice animals were incidental or normal background findings commonly reported in animals of this species and age.

The mean concentration–time profiles for males and females (data not shown) demonstrated that exposure to MB-102 increased with the increase in dose level from 60 to 600 μmol/kg. After intravenous bolus administration, MB-102 concentrations readily declined generally in a bi-exponential manner and with almost all concentrations below the lower limit of quantitation by 24 h. The mean half-life ($t_{1/2}$) values ranged from 0.693 to 0.767 h. Values for $t_{1/2}$ were generally dose independent. Values for mean clearance (CL) ranged from 0.222 to 0.247 L/h/kg and appeared dose independent. Values for mean volume at steady state (V_{ss}) ranged from 0.121 to 0.152 L/kg and also appeared dose independent. Values for mean V_{ss} were less than the total body water of a 10 kg dog but greater than the blood volume, indicating that MB-102 distributed throughout the extracellular space (0.276 L/kg) after intravenous administration.

No marked sex differences were observed in mean MB-102 maximum plasma concentration (C_{max}) and area under the plasma concentration–time curve ($AUC_{0\text{-}24}$) values, as the differences were less than twofold. The increases in mean C_{max} and $AUC_{0\text{-}24}$ for males and females were generally dose proportional.

Very few notable differences for clinical pathology test results (Tables 15A and B and 16A and B) were observed between control and treated animals, and all were consistent with normal variation and considered incidental. No unscheduled deaths occurred. Significant changes in body and organ weights for the dosing phase interim and final sacrifices were not found. Microscopic observations upon sacrifice were made on many tissues including bone, spinal cord, nerve, pituitary, adrenal, aorta, trachea, thyroid, parathyroid, lung, liver, gallbladder, kidney, bladder, esophagus, stomach, duodenum, colon, ileum, jejunum, cecum, rectum, pancreas, spleen, thymus, muscle, tongue, mammary, prostate, testis, epididymis, ovary, uterus, vagina, eye, heart, and brain. Microscopic findings related to the test article were not found. A variety of observations were recorded but were considered incidental for this age, sex, and species of animal.

3.10. Single dose cardiovascular safety pharmacology study in dogs

This study evaluated the potential effects of a single intravenous bolus administration of 0, 60, 200, or 600 μmol/kg MB-102 on the cardiovascular system in the dog. The measurements provided an evaluation of potential effects on blood pressure, heart rate, and electrocardiogram as well as body

Table 14
Mean motor activity: 1–45 min post dose (males and females).

Group	DT (cm)	RT (sec)	ST (sec)	AT (sec)	BSM	HC	AC	V1C	V1B
Males									
Group 1 0 μmol/kg	1272.1 ± 521.6	432.4 ± 121.8	281.0 ± 62.9	186.6 ± 63.7	146.0 ± 32.7	1409.5 ± 493.0	829.3 ± 363.8	121.0 ± 90.9	23.1 ± 13.8
Group 2 180 μmol/kg	1508.6 ± 505.4	354.7 ± 85.8	331.2 ± 35.7	214.1 ± 59.8	161.4 ± 20.9	1743.5 ± 375.4	1050.9 ± 313.0	165.6 ± 90.5	29.7 ± 16.7
Group 3 600 μmol/kg	1361.8 ± 653.9	422.0 ± 139.5	284.8 ± 64.4	193.2 ± 81.2	143.1 ± 29.6	1568.7 ± 635.1	949.5 ± 467.1	150.2 ± 106.0	33.1 ± 22.6
Group 4 1200 μmol/kg	864.3 ± 486.7	478.6 ± 120.5	290.3 ± 71.5	131.1 ± 66.2	134.1 ± 29.3	1165.2 ± 434.5	579.0 ± 312.2	121.0 ± 90.9	23.1 ± 13.8
Females									
Group 1 0 μmol/kg	1891.9 ± 718.5	348.8 ± 107.4	313.5 ± 53.1	237.7 ± 81.0	154.3 ± 25.8	2262.3 ± 566.2	1477.2 ± 494.0	254.2 ± 107.6	44.1 ± 16.3
Group 2 180 μmol/kg	1801.2 ± 699.1	393.8 ± 104.9	290.7 ± 53.5	215.5 ± 74.7	146.1 ± 18.8	2021.3 ± 637.4	1333.0 ± 535.9	247.4 ± 143.0	44.3 ± 21.0
Group 3 600 μmol/kg	2050.0 ± 985.4	354.1 ± 113.8	303.2 ± 50.1	242.7 ± 84.8	152.2 ± 24.1	2339.0 ± 995.0	1590.4 ± 841.8	254.0 ± 144.4	44.5 ± 20.0
Group 4 1200 μmol/kg	1616.3 ± 685.0	414.1 ± 99.1	282.7 ± 45.7	203.2 ± 64.6	143.2 ± 22.5	1826.5 ± 666.4	1187.5 ± 525.5	183.1 ± 99.1	34.4 ± 15.6

Note: All values are mean ± standard deviation. *Abbreviations used:* sec, seconds; DT, distance traveled; RT, resting time; ST, stereotypic time; AT, ambulatory time; BSM, bursts of stereotypic movement; HC, horizontal counts; AC, ambulatory counts; V1C, vertical counts; V1B, vertical breaks.

Table 15A
Hematological parameters from male beagle dogs (n = 4/group) 3 days post dosing of MB-102.

Parameter	Dose (μmol/kg)			
	0 μmol/kg	60 μmol/kg	200 μmol/kg	600 μmol/kg
RBC (e6/μL)	6.36 ± 0.274	5.96 ± 0.329	6.06 ± 0.244	6.06 ± 0.211
WBC (e5/μL)	11.48 ± 1.60	12.40 ± 2.28	11.81 ± 2.45	10.31 ± 1.30
Baso (e3/μL)	0.07 ± 0.050	0.09 ± 0.041	0.07 ± 0.026	0.04 ± 0.015
Mono (e3/μL)	0.66 ± 0.238	0.73 ± 0.125	0.61 ± 0.155	0.42 ± 0.108
Luc (e3/μL)	0.06 ± 0.031	0.05 ± 0.015	0.05 ± 0.010	0.03 ± 0.010
Eos (e3/μL)	0.20 ± 0.121	0.15 ± 0.034	0.21 ± 0.097	0.14 ± 0.017
Neut (e3/μL)	7.23 ± 0.662	8.03 ± 1.802	8.05 ± 1.895	6.79 ± 1.230
Lym (e3/uL)	3.27 ± 0.770	3.34 ± 0.449	2.83 ± 0.389	2.93 ± 0.487
Plt (e3/μL)	404 ± 86.7	370 ± 39.4	450 ± 78.7	411 ± 30.3
HGB (g/dL)	13.8 ± 0.64	12.5 ± 0.68	12.7 ± 0.40	12.7 ± 0.67
Hct (%)	43.6 ± 2.06	39.8 ± 2.39	40.4 ± 0.84	40.5 ± 1.87
[a]PT (s)	9.3 ± 0.13	9.4 ± 0.31	9.5 ± 0.34	9.5 ± 0.39
[a]APTT (s)	10.4 ± 0.47	11.2 ± 1.09	11.0 ± 0.67	10.6 ± 0.38

Note: All values are mean ± standard deviation. *Abbreviations used:* RBC, red blood cell count; WBC, white blood cell count; Baso, basophil granulocytes; Mono, monocytes; Luc, large unstained cells; Eos, eosinophil granulocytes; Neut, neutrophil granulocytes; Lym, lymphocytes; Plt, platelets; HGB, hemoglobin; Hct, hematocrit; PT(s), prothrombin time; APTT(s), activated partial thromboplastin time.
[a] PT and APTT performed on Day 3 post-dosing, n = 4/group.

Table 15B
Hematological Parameters from female Beagle dogs (n = 4/group) 3 days post dosing of MB-102.

Parameter	Dose (μmol/kg)			
	0 μmol/kg	60 μmol/kg	200 μmol/kg	600 μmol/kg
RBC (e6/μL)	6.73 ± 0.217	6.83 ± 0.336	6.57 ± 0.8.3	6.53 ± 0.210
WBC (e5/μL)	9.61 ± 0.387	9.45 ± 1.194	12.14 ± 1.763	12.56 ± 1.73
Baso (e3/μL)	0.07 ± 0.036	0.09 ± 0.021	0.09 ± 0.014	0.06 ± 0.024
Mono (e3/μL)	0.41 ± 0.101	0.48 ± 0.083	0.60 ± 0.332	0.70 ± 0.242
Luc (e3/μL)	0.05 ± 0.017	0.06 ± 0.015	0.06 ± 0.013	0.04 ± 0.017
Eos (e3/μL)	0.20 ± 0.070	0.15 ± 0.031	0.20 ± 0.059	0.19 ± 0.115
Neut (e3/μL)	5.99 ± 0.792	6.11 ± 1.095	7.61 ± 0.454	8.89 ± 1.503
Lym (e3/μL)	2.90 ± 0.510	2.57 ± 0.640	3.60 ± 1.123	2.70 ± 0.387
Plt (e3/μL)	330 ± 42.0	348 ± 51.9	410 ± 62.9	332 ± 56.2
HGB (%)	14.1 ± 0.47	14.6 ± 0.50	14.0 ± 1.50	13.8 ± 1.02
Hct (%)	44.4 ± 1.06	45.8 ± 1.86	44.1 ± 4.34	43.5 ± 2.52
[a]PT (s)	9.6 ± 0.80	9.5 ± 0.17	9.7 ± 0.25	9.5 ± 0.14
[a]APTT (s)	11.0 ± 0.44	10.6 ± 0.58	10.8 ± 0.45	10.9 ± 0.57

Note: All values are mean ± standard deviation. *Abbreviations used:* RBC, red blood cell count; WBC, white blood cell count; Baso, basophil granulocytes; Mono, monocytes; Luc, large unstained cells; Eos, eosinophil granulocytes; Neut, neutrophil granulocytes; Lym, lymphocytes; Plt, platelets; HGB, hemoglobin; Hct, hematocrit; PT(s), prothrombin time; APTT(s), activated partial thromboplastin time.
[a] PT and APTT performed on Day 3 post-dosing, n = 4/group.

temperature. No animals died or were deemed moribund during the study. Test article-related clinical observations were limited to one dog following the 600 μmol/kg dose that became limp immediately after administration of MB-102. The animal recovered within 20 min and did not exhibit any further test article-related observations.

Blood Pressure parameters were significantly lower from 5 to 20 min (systolic pressure) and from 5 to 25 min (diastolic and mean pressure) postdose for the high dose group, 600 μmol/kg MB-102. Fig. 3A shows the mean blood pressure results recorded from 0 to 24 h. Fig. 3B captures the changes in mean blood pressure from −0.25 h through 1.0 h post dose. There was one, single incidence of markedly low blood pressure immediately postdose in one of four dogs receiving 600 μmol/kg MB-102 with associated clinical signs of listlessness, as mentioned above. This decrease in blood pressure was considered adverse; however, the effect was of short duration (resolution within 25 min of dose completion) and produced no long-term adverse effects (Fig. 3A and B). The data show the single event occurring shortly administration followed by full recovery of the animal after approximately 20 min. These group mean changes observed in the high dose animals were the result of one dog that experienced a transient, marked drop in blood pressure during the 20-min postdose period with subsequent full recovery of the animal to normal values for the remainder (24 h) of the study. As a result of this single event, the NOEL in dogs has to be reported as less than the highest dose administered.

Heart Rate (HR) and ECG intervals had some significant findings in the 5- to 30-min postdose period but these were not considered test article-related findings since primary changes in HR during this period were due to excitation related to dose administration techniques and changes in the ECG parameters were considered related to heart rate change. Representative QT Interval graphs are shown in Fig. 4A and B. Fig. 4A represents the QT intervals recorded from 0 to 24 h post dosing. Fig. 4B shows the shows the QT intervals recorded from −0.25 h to 1.0 h post dosing. Body temperatures were not affected by administration of MB-102. There were no other test article-related effects within the physiological

Table 16A
Clinical chemistry parameters from male beagle dogs (n = 4/group) 3 days post dosing of MB-102.

Parameter	Dose (μmol/kg)			
	0 μmol/kg	60 μmol/kg	200 μmol/kg	600 μmol/kg
Glu (mmol/L)	105 ± 2.5	100 ± 1.6	105 ± 4.6	105 ± 11.0
Alb (g/L)	3.4 ± 0.13	3.2 ± 0.10	3.4 ± 0.05	3.3 ± 0.05
Chol (mmol/L)	184 ± 26.1	153 ± 6.1	173 ± 17.3	182 ± 29.7
Crea (μmol/L)	0.8 ± 0.06	0.8 ± 0.10	0.8 ± 0.06	0.8 ± 0.06
TBIL (μmol/L)	0.1 ± 0.00	0.01 ± 0.00	0.1 ± 0.00	0.1 ± 0.00
Phos (mg/dL)	6.2 ± 0.5	6.0 ± 0.14	6.2 ± 0.46	5.9 ± 0.43
TP (g/dL)	5.4 ± 0.25	5.5 ± 0.21	5.6 ± 0.25	5.3 ± 0.18
UN (mg/dL)	11.0 ± 1.5	12.0 ± 1.7	11.0 ± 0.8	11.0 ± 1.0
GLOB (g/L)	2.1 ± 0.30	2.2 ± 0.13	2.1 ± 0.26	2.0 ± 0.17
AGR	1.6 ± 0.25	1.5 ± 0.05	1.6 ± 0.19	1.6 ± 0.14
ALP (U/L)	74 ± 19.5	57 ± 4.40	99 ± 24.6	68 ± 18.9
AST (U/L)	27 ± 5.4	30 ± 5.8	29 ± 5.4	30 ± 5.3
ALT (U/L)	29 ± 7.5	24 ± 6.0	24 ± 1.6	25 ± 6.2
Na^+ (mmol/L)	147 ± 1.3	146 ± 0.60	146 ± 0.50	145 ± 1.00
K^+ (mmol/L)	4.4 ± 0.13	4.1 ± 0.28	4.4 ± 0.28	4.1 ± 0.12
Ca^{++} (mmol/L)	11.3 ± 0.39	11.1 ± 0.24	11.2 ± 0.29	11.0 ± 0.28
Cl^- (mmol/L)	112 ± 1.3	111 ± 1.9	112 ± 1.7	111 ± 1.6

Note: All values are mean ± standard deviation. *Abbreviations used:* Glu, glucose; Alb, albumin; Chol, cholesterol; Crea, creatinine; TBIL, total bilirubin; Phos, phosphate; TP, total protein; UN, blood urea nitrogen; GLOB, globulins; AGR, albumin-globulin ratio; ALP, alkaline phosphatase; AST, aspartate transaminase; ALT, alanine transaminase; Na^+, sodium; K^+, potassium; Ca^{++}, calcium; Cl^-, chloride.

Table 16B
Clinical Chemistry Parameters from female beagle dogs (n = 4/group) 3 days post dosing of MB-102.

Parameter	Dose (μmol/kg)			
	0 μmol/kg	60 μmol/kg	200 μmol/kg	600 μmol/kg
Glu (mmol/L)	101 ± 2.6	102 ± 8.8	97 ± 5.5	96 ± 8.0
Alb (g/L)	3.5 ± 0.05	3.4 ± 0.10	3.5 ± 0.13	3.4 ± 0.13
Chol (mmol/L)	171 ± 15.6	170 ± 11.5	179 ± 13.0	167 ± 25.6
Crea (μmol/L)	0.8 ± 0.05	0.8 ± 0.05	0.9 ± 0.06	0.8 ± 0.10
TBIL (μmol/L)	0.1 ± 0.0	0.01 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Phos (mg/dL)	5.9 ± 0.17	5.9 ± 0.44	6.5 ± 0.69	6.3 ± 0.87
TP (g/L)	5.4 ± 0.25	5.5 ± 0.21	5.6 ± 0.25	5.3 ± 0.18
UN (mg/dL)	12.0 ± 1.5	12.0 ± 0.8	14.0 ± 2.2	14.0 ± 2.2
GLOB (g/L)	2.0 ± 0.22	2.1 ± 0.28	1.8 ± 0.13	2.0 ± 0.22
AGR	1.8 ± 0.17	1.7 ± 0.21	1.9 ± 0.18	1.7 ± 0.19
ALP (U/L)	74 ± 20.8	62 ± 16.6	104 ± 22.7	75 ± 13.8
AST (U/L)	32 ± 7.7	32 ± 9.7	35 ± 11.4	32 ± 5.3
ALT (U/L)	27 ± 2.2	24 ± 1.7	29 ± 2.0	27 ± 3.0
Na^+ (mmol/L)	145 ± 0.8	146 ± 1.0	146 ± 0.50	146 ± 0.50
K^+ (mmol/L)	4.3 ± 0.37	4.2 ± 0.17	4.2 ± 0.29	4.2 ± 0.13
Ca^{++} (mmol/L)	11.3 ± 0.29	11.1 ± 0.19	11.2 ± 0.22	11.0 ± 0.24
Cl^- (mmol/L)	111 ± 0.06	111 ± 1.8	110 ± 1.5	111 ± 0.8

Note: All values are mean ± standard deviation. *Abbreviations used:* Glu, glucose; Alb, albumin; Chol, cholesterol; Crea, creatinine; TBIL, total bilirubin; Phos, phosphate; TP, total protein; UN, blood urea nitrogen; GLOB, globulins; AGR, albumin-globulin ratio; ALP, alkaline phosphatase; AST, aspartate transaminase; ALT, alanine transaminase; Na^+, sodium; K^+, potassium; Ca^{++}, calcium; Cl^-, chloride.

parameters measured. Additionally, the ECG tracings underwent a further cardiovascular evaluation. All dogs maintained sinus rhythms (sinus rhythm or sinus arrhythmia) throughout the study. All 4 dogs had rare single atrial premature depolarizations. This was considered a normal variant. No ventricular premature depolarizations were noted. No consistent morphology changes were detected on any traces.

Thus, intravenous dosing with MB-102 at up to 600 μmol/kg (223.4 mg/kg) did not have any toxicologic effects on cardiac rhythm or ECG morphology in dogs in this study.

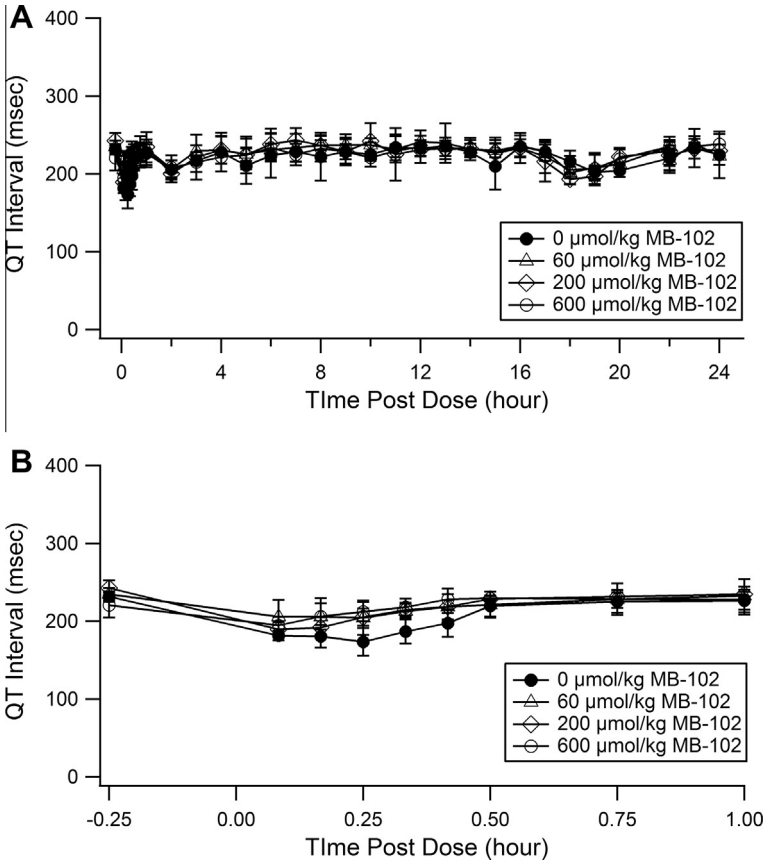

Fig. 3. Mean blood pressure results from 0-24 hours (A) and from -0.25 hours through 1 hour (B) post dosing of MB-102 in beagle dogs.

4. Discussion and conclusion

A series of safety/pharmacology studies, both *in vitro* and *in vivo*, were designed and conducted on MB-102, a novel fluorescent tracer that has been developed for the real-time determination of glomerular filtration rate (GFR). These studies focused on a battery of tests necessary for regulatory clearance in preparation of the first evaluation of MB-102 in a human clinical setting.

The *in vitro* studies included (1) hemolytic potential and blood compatibility, (2) bacterial reverse mutation assay, (3) chromosomal aberration assay, (4) CYP-450 enzyme assay and (5) hERG channel changes. When mixed with human blood no demonstrable hemolysis was observed from 25 to 100 μM concentrations of MB-102. Additionally, there was no observation of any flocculation or precipitation when admixed with human plasma. When MB-102 was evaluated in 5 different bacterial test strains at concentrations reaching 5000 μg/plate, there was no significant increase in revertants/plate either in the presence or absence of metabolic activation with S9, indicating no mutagenic potential for the test article. When screened in a panel of 5 enzyme systems and 68 receptor assays for abnormal activation or inhibition of those biochemical markers, no significant findings were noted for any of these common receptor types. Furthermore, when screened for the potential to induce chromosomal aberrations in human lymphocytes, no significant increase in the mitotic index was observed for MB-102 at concentrations of 10 mM with or without metabolic stimulation of the cultures. Finally, when evaluated in the hERG channel assay, at 10 μM MB-102 did not induce a significant change in the hERG channel potential. The assay was repeated at 300 μM concentration and again this resulted in no significant change in the hERG channel potential. Thus, the IC_{50} for the inhibition potential of MB-102 could not be calculated from this assay.

In vivo, MB-102 was first evaluated in a series of safety/pharmacology studies in rodents. Included in these studies were (1) single dose intravenous safety/toxicity study, (2) a single dose CNS safety study and (3) and a single dose respiratory safety study. The results of the single dose toxicity study resulted in no significant changes in food consumption, body weight, body temperature or organ weights. All animals in this study survived to scheduled sacrifice timepoints. No significant microscopic or macroscopic changes were noted upon autopsy. Hematologic and clinical chemistry parameters were largely (except as noted) not significant and all differences were consistent with normal variation. The pharmacokinetic parameters of half-life ($t_{1/2}$), steady state volume (V_{ss}), and clearance (Cl), were all dose independent. The only clinical observation noted in this study was a transient yellow discoloration of in all animals treated with MB-102. Most commonly this discoloration occurred in the paws, ears and nose areas, and also resulted in discolored urine. More pronounced discoloration was noted in the two higher dose groups corresponding to 200 times the expected human dose. In all cases the discoloration disappeared within 24 h post administration. This effect was not considered to be clinically adverse as it was deemed to be the result of the nature of the colored test article. In the CNS safety study, the rodents were evaluated using both formal observation battery (FOB) and motor activity tests. In both male and female rats no significant changes in the FOB parameters were noted including gait abnormalities, involuntary motor movements, touch or auditory response. All motor activity parameters were also not significantly different as compared to control treated animals. When animals

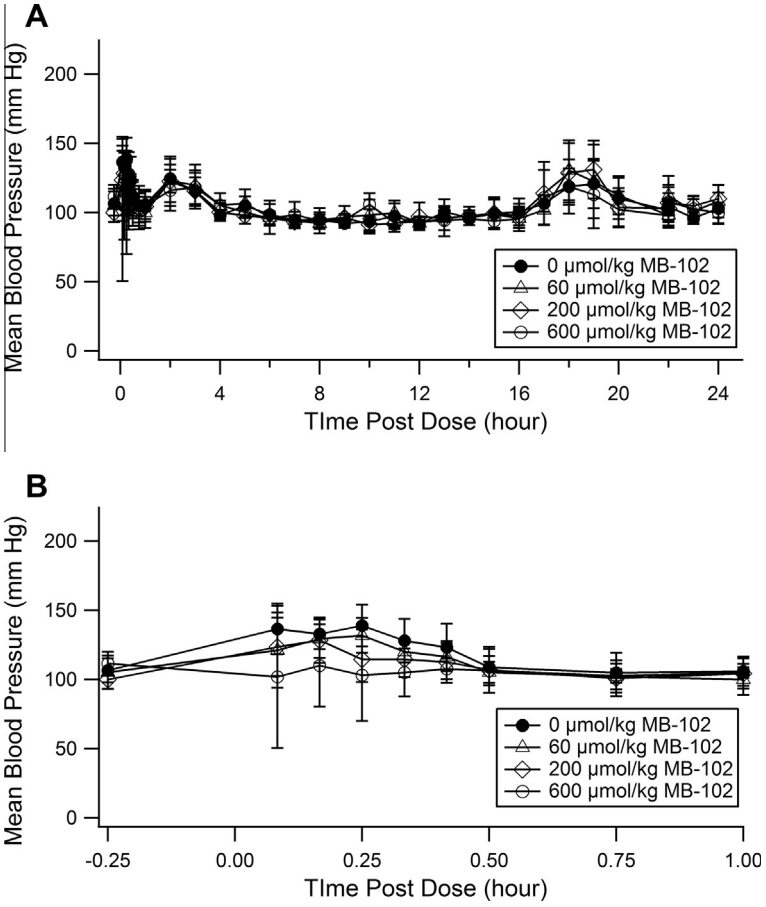

Fig. 4. QT interval data from 0-24 hours (A) and from -0.25 hours through 1 hour (B) post dosing of MB-102 in beagle dogs.

were treated with MB-102 in the single dose respiratory safety study, significant changes in respiratory rate (RR) and tidal volume (TV) were observed; however, these changes were not considered to be related to the test article as the findings did not correlate to dose levels or other trends following the administration of MB-102.

In beagle dogs, MB-102 was further evaluated in a single dose safety/toxicity study and in a single dose cardiovascular safety study. As previously observed in rodents, all treated animals survived to scheduled sacrifice timepoints, and no significant changes in body weight, body temperature, food consumption, or organ weights were noted. No significant changes in the hematologic or clinical chemistry parameters were observed at any dose levels (male or female) of MB-102. The pharmacokinetic parameters of half-life ($t_{1/2}$), steady state volume (V_{ss}), and clearance (Cl), were all dose independent. Transient yellow discoloration at various anatomical sites (i.e. gums, eyes, ears) was noted at the two higher dose levels; however, by 3 h post administration coloration returned to normal. Yellow discoloration of urine samples was also noted, particularly in the two higher dose groups. Again, this was deemed to be the result of the colored nature of the test article and not deemed adverse. Lastly, in the single dose cardiovascular safety study, the clinical observations were limited to a single animal following a high dose (600 μmol/kg) administration of MB-102. This animal exhibited a significant drop in blood pressures (both diastolic and systolic) lasting ~5–20 min post administration, and was considered to be adverse. The effects were transient; however, with the animal showing a full recovery at 25 min and demonstrating no further effects for the duration of the study (24 h). No other ECG or heart rate changes were observed.

In conclusion, MB-102 has been evaluated in a series of *in vitro* and *in vivo* safety/toxicity studies. The *in vitro* assays demonstrated a clean toxicity profile relating to potential hemolytic effects, mutation effects, chromosomal aberration effects, receptor activation or inhibition effects or hERG channel changes. *In vivo*, doses ranging from 200 to 300 times the estimated human dose were evaluated in both rodents and dogs assessing potential overall safety/toxicity, CNS and cardiovascular safety studies. In rats the NOEL was determined to be greater than 1200 μmol/kg and in dogs the NOEL was determined to be at least 200 μmol/kg. The accumulated results of these studies suggest that the safety/toxicity profile of MB-102 should be sufficient to begin a first-in-human clinical study.

Further nonclinical testing related to biodistribution and developmental toxicity will be performed following completion of the first-in-human clinical studies.

Conflict of interest

The authors declare that there is no conflict of interest.

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