

Discovery and Clinical Evaluation of MK-8150, A Novel Nitric Oxide Donor With a Unique Mechanism of Nitric Oxide Release

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Background—Nitric oxide donors are widely used to treat cardiovascular disease, but their major limitation is the development of tolerance, a multifactorial process to which the *in vivo* release of nitric oxide is thought to contribute. Here we describe the preclinical and clinical results of a translational drug development effort to create a next-generation nitric oxide donor with improved pharmacokinetic properties and a unique mechanism of nitric oxide release through CYP3A4 metabolism that was designed to circumvent the development of tolerance.

Methods and Results—Single- and multiple-dose studies in telemetered dogs showed that MK-8150 induced robust blood-pressure lowering that was sustained over 14 days. The molecule was safe and well tolerated in humans, and single doses reduced systolic blood pressure by 5 to 20 mm Hg in hypertensive patients. Multiple-dose studies in hypertensive patients showed that the blood-pressure-lowering effect diminished after 10 days, and 28-day studies showed that the hemodynamic effects were completely lost by day 28, even when the dose of MK-8150 was increased during the dosing period.

Conclusions—The novel nitric oxide donor MK-8150 induced significant blood-pressure lowering in dogs and humans for up to 14 days. However, despite a unique mechanism of nitric oxide release mediated by CYP3A4 metabolism, tolerance developed over 28 days, suggesting that tolerance to nitric oxide donors is multifactorial and cannot be overcome solely through altered *in vivo* release of nitric oxide.

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The nitric oxide (NO) donors are a class of vasorelaxant drugs that have been used for over a century in the treatment of cardiovascular disease. Commercially available, orally administered organic nitrate NO donors such as nitroglycerin (NTG), isosorbide mononitrate (ISMN), and isosorbide dinitrate (ISDN) are indicated for the treatment of angina. The general mechanism of action for NO involves stimulation of soluble guanylate cyclase (sGC), subsequent formation of cyclic GMP (cGMP), and consequent activation of protein kinase G, which downstream leads to vascular smooth

muscle relaxation. Clinically, this vasorelaxation leads to decreased preload, afterload, blood pressure (BP), and propensity for cardiac vasospasm as well as dilation of large coronary arteries. NO is also known to have anti-inflammatory and antithrombotic effects, although the clinical relevance of these properties is not well characterized.¹⁻⁴

Despite the critical importance of NO to regulation of cardiovascular physiology, the use of organic nitrate NO donors for the management of chronic cardiovascular disease has been limited, primarily due to the development of tolerance to the clinical and physiological effects of these compounds upon continuous administration. Although long-term administration of organic nitrates is utilized in selected patients, a nitrate-free period must be employed to avoid the development of tolerance, a strategy that can lead to fluctuations in BP control and/or increased incidence of symptoms and cardiac ischemic events.⁵⁻⁷ The mechanism of tolerance has been thoroughly investigated but remains elusive and highly debated,⁸ although metabolism and bioconversion to NO have been shown to play an important role.⁹ In addition to inducing tolerance, organic

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nitrate NO donors also have a notable side effect profile that includes headaches, dizziness, and hypotension. Clinical studies suggest that slower onset of action and reduced peak-to-trough ratio of BP effects can help mitigate the severity of such symptoms.

Based on the clinical limitations of currently available organic nitrates, our group perceived an unmet medical need for an NO donor that (1) does not induce tolerance, (2) has reduced peak-to-trough pharmacokinetic (PK) and pharmacodynamic (PD) ratios, and (3) has a slower onset of action compared with currently available organic NO donors. We hypothesized that a molecule undergoing unique metabolic release of NO *in vivo* would potentially circumvent the development of tolerance. Initially, we identified a novel chemical series of *O*²-alkylated diazeniumdiolate NO donors that had hitherto been studied but, to the best of our knowledge, had not yet advanced into clinical trials involving hypertensive patients.¹⁰ We postulated that the mechanism of NO release from these molecules would be unique in relation to that of organic nitrates in that it would occur slowly through cytochrome P450 metabolism and therefore, potentially overcome tolerance by offering improved kinetics. Additionally, early preclinical studies with these molecules showed improved PK properties, extended duration of action, and minimal peak-to-trough ratio, and we envisioned that these properties could potentially translate to an improved tolerability profile relative to that of existing organic nitrates. The present report describes the preclinical and clinical development of the diazeniumdiolate NO donor MK-8150.

Methods

Preclinical Studies

Chemistry

The novel *O*²-alkylated diazeniumdiolate derivatives MK-8150 ((*Z*)-3-(*tert*-butyl)-1-((1-(5-cyanopyridin-2-yl)piperidin-4-yl)oxy)triaz-1-ene 2-oxide; Figure 1) and Compound 2 ((*Z*)-3-(*tert*-butyl)-1-((1-(5-chloropyridin-2-yl)piperidin-4-yl)oxy)triaz-1-ene 2-oxide; Figure 1) were synthesized according to published procedures.¹¹

In Vitro and *In Vivo* Drug Metabolism Studies

Radiolabeled MK-8150, designated [¹⁴C]MK-8150, was used in preclinical studies for quantitative purposes. [¹⁴C]MK-8150 was incubated in liver-derived matrices (microsomes, hepatocytes) from animals and humans, samples were processed to precipitate the protein, and supernatant solutions were analyzed using liquid chromatography-high resolution mass spectrometry (LC-HRMS) to identify and quantify all drug-related material. In addition, the metabolic enzymes

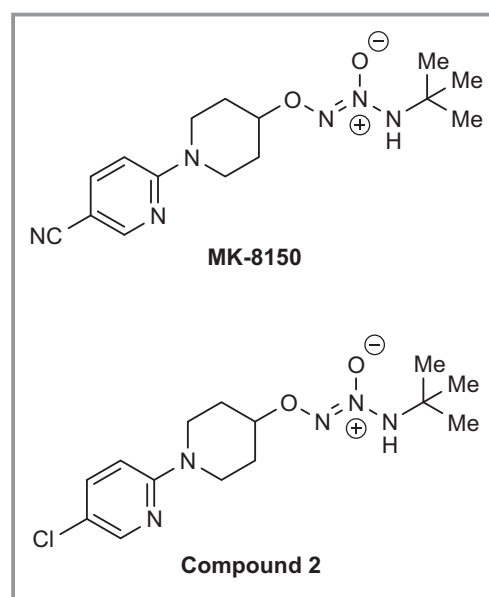


Figure 1. Chemical structures of MK-8150 and Compound 2.

responsible for the release of NO from [¹⁴C]MK-8150 were determined using coincubation of MK-8150 with chemical inhibitors of cytochrome P450 isoforms in plated hepatocytes and with individual recombinant cytochrome P450 isozymes.

Pharmacokinetic (PK) studies were conducted in rats and dogs using 1 mg/kg IV infusion of MK-8150, and plasma samples were collected over a 48-hour period to characterize the PK profile. Elimination pathways in bile-duct-cannulated rats and dogs were investigated following a single 5 mg/kg oral dose of [¹⁴C]MK-8150. Metabolic profiles were generated from bile and urine samples collected over 72 hours, and structures were assigned using LC-HRMS.

In Vitro cGMP Production in Primary Kidney Cell Line

Human renal proximal tubule cells (RPTEC; cat# CC-2553) and complete growth media (cat# CC-3190) were purchased from Lonza (Allendale, NJ). The cyclic GMP (cGMP) EIA kit was purchased from GE Healthcare (Piscataway, NJ; cat# RPN-226). The HTRF cGMP Assay kit (cat#62GM2PEC) was purchased from Cisbio (Bedford, MA). 3-Isobutyl-1-methylxanthine (IBMX, cat# 17018) was purchased from Sigma (St. Louis, MO). Cells were cultured at 37°C in a 5% CO₂ incubator following the vendor protocol. Cells were seeded in 96-well plates at 20 000 cells/well in 0.1 mL complete media overnight. The next day, the media was replaced with fresh complete media plus 0.5 mmol/L IBMX and the cells were treated for 48 hours or as indicated in Figure 2. At the end of the treatment, the cells were harvested and the supernatant collected. A 100-μL aliquot of Lysis Reagent 1 provided in the cGMP EIA kit, plus 0.5 mmol/L IBMX, was added to each well to lyse cells for the intracellular cGMP measurement. The protocol from the EIA kit

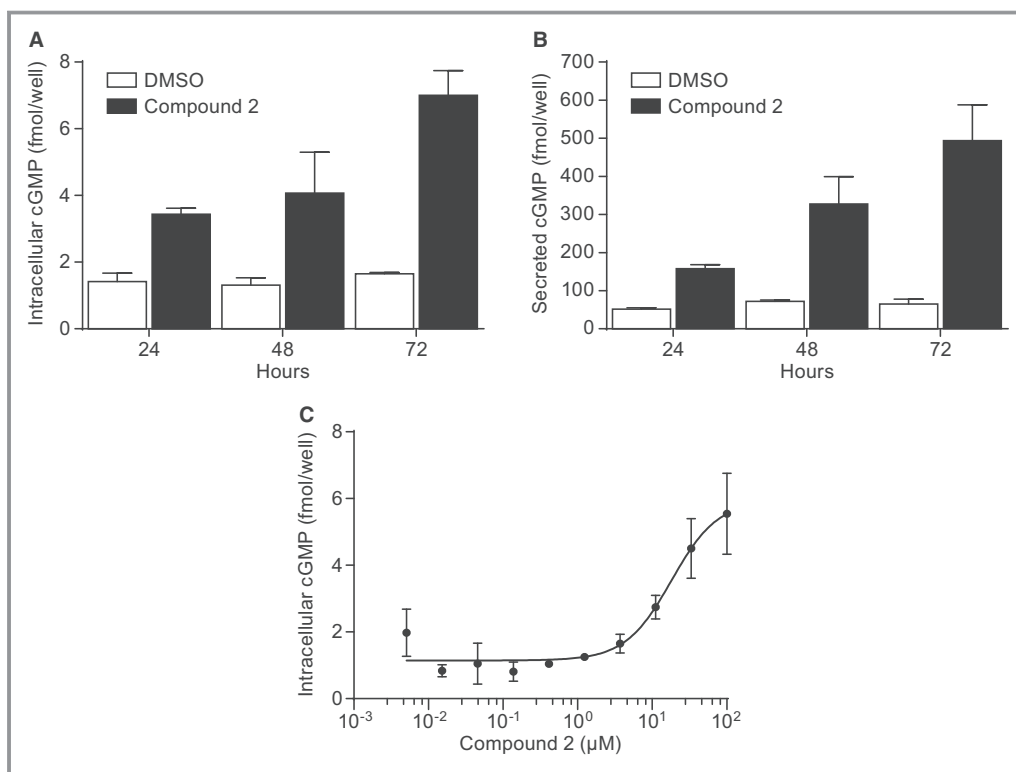


Figure 2. Effect of Compound 2 on cGMP in human primary renal proximal epithelial tubule cells. A, Effect of Compound 2 compared with DMSO vehicle on intracellular cGMP over time. B, Effect of Compound 2 compared with DMSO vehicle on secreted cGMP over time. C, Relationship between Compound 2 concentration and intracellular cGMP level. Values are expressed as group means \pm SE. DMSO indicates dimethyl sulfoxide.

was followed to determine intracellular cGMP levels. The levels of secreted cGMP in the supernatant were determined by the HTRF method following the kit instructions.

In Vivo Canine Studies

The effects of MK-8150 on arterial BP and heart rate (HR) were evaluated in telemeterized conscious beagle dogs using both single- and multiple-dose regimens. Prior to dosing, BP and HR were recorded for 24 hours to derive baseline values and to sort animals into BP-matched groups. In the single-dose study, 3 groups (n=6 per group) of male adult beagle dogs (10.8–14.6 kg) were given a dose of vehicle by oral gavage. Forty-eight hours later, 1 group of animals was given a second dose of vehicle as control, while the other 2 groups were treated with either 1.0 or 3.0 mg/kg of MK-8150. In the multiple-dose study, 2 groups (n=6 per group) of male adult beagle dogs (10.4–14.2 kg) were administered a dose of vehicle on day 0 by oral gavage. During the next 14 days, from day 1 through day 14, 1 group of animals continued to receive once-daily vehicle as control, while the other group was treated with once-daily MK-8150 at 3.0 mg/kg. Trough PK measurements at 24 hours post-drug administration were obtained on days 1, 5, 8, 11, and 14. MK-8150 was formulated in a mixed vehicle composed of

70% polyethylene glycol 400 (PEG 400) and 30% H₂O (v/v) and was administered at a volume of 1.0 mL/kg in both the single-treatment and 14-day studies.

In the single-dose study, the pressure sensor of the telemetry device was placed in the upper thoracic aorta per device design to measure central BP, whereas in the 14-day study, the pressure catheter was placed in a femoral artery to record peripheral BP. In both cases, BP signals were recorded continuously throughout the course of the study using device-specific data acquisition software (CA Recorder, DISS LLC, Dexter, MI; or DataQuest ART, DSI, New Brighton, MN). Treatment-related effects were defined as changes from baseline and were calculated by subtracting the baseline values from the corresponding values obtained following treatment administration. The significance level was set at $P < 0.05$. All values are expressed as mean \pm SEM.

Clinical Studies

A single-ascending-dose (SAD; PN 001; clinicaltrials.gov number NCT01590810) study and a multiple-dose (MD; PN 002; clinicaltrials.gov number NCT01656408) study were conducted in humans to assess the efficacy, safety,

tolerability, PK, and PD of MK-8150. All study subjects provided written informed consent prior to participating. Both studies were conducted in accordance with principles of Good Clinical Practice and were approved by the appropriate institutional review boards and/or ethics review committees.

The studies enrolled healthy, normotensive (systolic BP [SBP] of >110 and ≤ 140 mm Hg), male subjects 18 to 50 years of age, and male patients 18 to 65 years of age with hypertension (SBP of 135–175 mm Hg and diastolic BP [DBP] of 85–105 mm Hg on at least 3 different occasions at the screening visit). Major exclusion criteria included estimated creatinine clearance ≤ 80 mL/min, history of any illness that might confound the results of the study or pose an additional risk to the subject, and anticipated use of phosphodiesterase inhibitors, organic nitrates, or cytochrome P450 3A4 inhibitors or inducers during the course of the study.

Plasma PK parameters were determined after oral administration of single and multiple doses of MK-8150. Heart rate and peripheral (cuff) SBP (pSBP) and DBP (pDBP) were evaluated at selected time points using a validated automatic BP device. The safety and tolerability of MK-8150 were monitored by clinical assessment of adverse experiences and by continuous or repeated measurements of vital signs, physical examinations, electrocardiograms (ECGs), and standard laboratory safety tests (hematology, chemistry and urinalysis). Laboratory evaluation also included erythrocyte sedimentation rate, high sensitivity C-reactive protein, cardiac-specific troponin, methemoglobin, and urine microalbumin. Cross-panel and individual hemodynamic stopping criteria were applied to further ensure subjects' safety.

Singe-Ascending-Dose Study in Hypertensive Patients

A randomized, double-blind, placebo-controlled, SAD study was performed in healthy male subjects and male patients with hypertension. Subjects received alternating single ascending oral doses of MK-8150 or placebo in up to 5 treatment periods. All doses were administered after an overnight fast, and there was at least a 3-day washout between treatment periods. The first 2 panels each enrolled 8 healthy subjects and were used primarily to establish safety, tolerability, and PK. The third panel enrolled 8 patients with hypertension who were either treatment naive or washed off of existing anti-hypertensive therapy for at least 14 days prior to randomization, and was used to determine safety and tolerability in the patient population as well as PD effects using the parameters listed above. A fourth panel of 10 healthy subjects tested higher doses up to 200 mg to establish a safety margin (data not shown).

Multiple-Dose Study in Hypertensive Patients

Following the SAD study, a randomized, multicenter, double-blind, placebo-controlled, MD study was performed in male patients with hypertension. A total of 104 patients were

enrolled and randomized across 10 panels to receive either MK-8150 or matching placebo; results from 6 of these panels are reported here. In each of the first 4 panels (1–4), 8 male patients with hypertension received once-daily MK-8150 ($n=6$) 5, 10, 15, or 20 mg, or placebo ($n=2$), for 10 consecutive days (days 1–10). In the fifth panel ($n=18$), 12 patients received MK-8150, 10 mg QD (days 1–7), followed by 20 mg QD (days 8–28), and 6 patients received matching placebo. In the sixth panel ($n=18$), 12 patients received MK-8150 5 mg QD (days 1–7), 10 mg QD (days 8–14), 20 mg QD (days 15–21), and 40 mg QD (days 22–28), and 6 patients received matching placebo. The remaining panels explored the effects of MK-8150 in elderly male/female hypertensive patients, male/female patients with resistant hypertension, combination with other antihypertensive medications, and healthy young men.

PK-PD Modeling and Simulation

Modeling and simulation were first used to aid in the transition of MK-8150 from preclinical candidate to clinical studies. Based on single- and multiple-dose clinical data, PK and PK/PD models were developed to characterize the exposure-response relationship and to select doses for the clinical studies. The PK model consisted of a standard 2-compartment model. Because BP exhibits circadian variations over a 24-hour period, a circadian rhythm model was developed using clinical data from these studies. Subsequently, a placebo model was used to describe the placebo data. Subjects with higher baseline BP were observed to have a larger drug effect, so a baseline BP covariate modulating the efficacy of the compound was incorporated; age covaried significantly with baseline BP. A direct-effect model was employed, and the drug effect was captured with a standard E_{\max} model. A modulation of the EC_{50} of the PK/PD relationship over time was used to describe the change in the PK/PD relationship that occurred over time in the MD studies.

Statistical Methods

Ordinary 1-way ANOVA and the Bonferroni multiple-comparison procedure were adopted in the single-dose canine study to detect significance between treatment groups. Repeated efficacy tests using 2-way ANOVA and the Dunnett multiple-comparison procedure were employed in the chronic canine study to compare changes between treatment and baseline within group and between vehicle and MK-8150-treated groups. Preclinical in vitro studies measuring increases in cGMP in response to Compound 2 were independently repeated a minimum of 3 times. Values are expressed as group means \pm SEM.

Clinical data analyzed on the log transformation were back-transformed to the original scale (exponentiated least-squares

means) when mean differences and corresponding standard errors were reported. Summary statistics and plots were generated for the change from baseline values, as deemed clinically appropriate for safety endpoints. All panels were included in the PD analyses, and placebo data (pd) were pooled across panels as appropriate (panels 1 to 4 were pooled, 5 and 6 were pooled, panels 9 and 10 were pooled). A trapezoidal method was used to calculate the area under the curve for each pd parameter; a PROC MIXED model (version SAS9.3) was used to assess each pd parameter with covariates of baseline, day, treatment and treatment-by-day interaction terms, and a repeated day term with a random subject term assuming compound symmetry covariance structure.

For each of the PD objectives (SBP and HR), the summary statistics for each dose was provided with a corresponding 90% confidence interval for the comparison of interest (each MK-8150 dose—placebo). Descriptive statistics were also provided by treatment and day for each PK parameter. Given the small sample size and main goal to estimate many of the PD measures of this early clinical compound, the *P*-values were not multiplicity-adjusted between parameters.

Results

MK-8150 is Primarily Metabolized by CYP3A4 to Yield NO

Analysis of metabolites following incubation of MK-8150 in cryo-preserved hepatocyte suspensions, NADPH-supplemented liver microsomal preparations, or plated hepatocytes (rat, dog, and human) indicated that NO was generated in all of these matrices. CYP3A4 was found to be the major enzyme responsible for production of NO using coinubation of MK-8150 with L-754,394, a known selective inhibitor of CYP3A4,¹² in plated hepatocytes. Additionally, when coinubated with a battery of recombinant CYPs (1A1, 1B1, 2A6, 2B6, 2C8, 2C9, 2C19, 2E1, 3A4), CYP3A4 was identified as the only isozyme responsible for catalyzing the metabolic reaction that generates NO (data not shown).

Metabolic profiles generated from bile and urine from rats and dogs indicated that ~70% and 30% of the MK-8150 dose, respectively, was converted to NO in these 2 species (data not shown).

Increased cGMP Production in a Primary Kidney Cell Line

To further investigate the mechanism of action in vitro, we identified primary cells that contained sGC and had robust CYP3A4 expression (data not shown). NO is notoriously difficult to measure directly because it is highly reactive;

however, NO activates sGC to produce cGMP, so increases in cGMP are considered downstream evidence of NO production. Renal proximal epithelial tubule cells were incubated with Compound 2, a close analogue of MK-8150, for various durations, and then cellular and secreted cGMP levels were measured. Incubation with Compound 2, but not dimethyl sulfoxide, produced a dose- and time-dependent increase in cGMP, as illustrated in Figure 2.

In Vivo Effects of MK-8150 in Canines

The hemodynamic effects of MK-8150 were evaluated in free-roaming, telemetered, normotensive beagle dogs following single and chronic (14-day) oral drug administration. The results of a single administration of MK-8150 at 1.0 and 3.0 mg/kg are shown in Table 1 and Figure 3. At the 3 mg/kg dose, significant lowering of SBP, DBP, mean BP (MBP), and pulse pressure (PP) as well as an increase in HR were observed. The BP-lowering effect reached a plateau in ~2 hours and lasted for up to 20 hours. The ratio of SBP peak response (2–4 hours after dosing) to trough response (18–20 hours after dosing) was <2, indicating sustained and relatively steady BP lowering. While the BP decrease was sustained for >24 hours, the HR increase lasted ~12 hours (data not shown).

Based on the potent hemodynamic effects of single MK-8150 doses, a second, 14-day canine study was performed to assess the durability of the hemodynamic effects. Daily administration of MK-8150 at 3 mg/kg for 14 days resulted in significant reductions in SBP that were sustained throughout the study (Figure 4). Daily HR was increased by an amount ranging from ~5 to 10 beats/min. Plasma concentrations of MK-8150 measured 24 hours after dosing on days 1, 5, 8, 11, and 14 ranged from 0.44±0.13 to 0.53±0.17 μmol/L, indicating consistent drug exposure

Table 1. Canine Studies

	Baseline		Change From Baseline	
	Vehicle	MK-8150	Vehicle	MK-8150
SBP, mm Hg	132±0.8	134±1.2	0.04±0.8	−19±0.9*
DBP, mm Hg	85±0.5	86±0.5	0.23±0.7	−3.4±1.0*
MBP, mm Hg	107±0.5	108±0.7	−0.5±0.5	−8.6±0.8*
PP, mm Hg	47±0.5	48±0.7	−0.4±0.6	−15.6±1.1*
HR, beats/min	73±1.6	70±1.5	−0.5±0.4	8.1±2.1*

DBP indicates diastolic blood pressure; HR, heart rate; MBP, mean blood pressure; PP, pulse pressure; SBP, systolic blood pressure.

Pretreatment baseline and 18-hour time-weighted average (TWA) changes in SBP, DBP, MBP, PP, and HR from pretreatment reference in 6 conscious beagle dogs treated with a single oral dose of MK-8150 3 mg/kg.

**P*<0.05, compared to vehicle group.

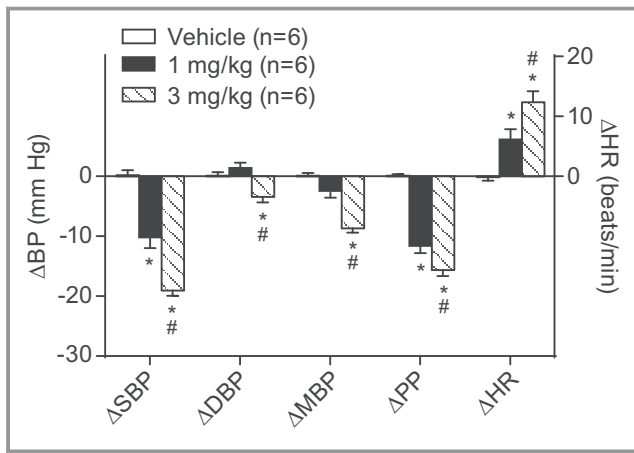


Figure 3. Eighteen-hour time-weighted average of change from baseline in systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP), pulse pressure (PP), and heart rate (HR) in 6 dogs treated with a single dose of vehicle or MK-8150 1 or 3 mg/kg. * $P < 0.05$, compared to vehicle control; # $P < 0.05$, compared to 1 mg/kg.

throughout the course of study, with no accumulation of MK-8150 (data not shown).

Pharmacokinetics of MK-8150 in Humans

MK-8150 displayed dose-linear, time-independent PK across the dose ranges tested in the SAD and MD studies (Figure 5).

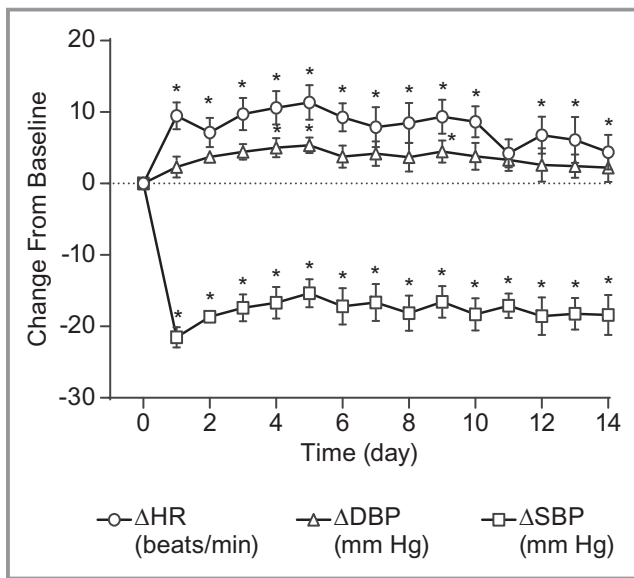


Figure 4. Daily average of change from baseline in heart rate (HR), diastolic blood pressure (DBP), and systolic blood pressure (SBP) in 6 dogs treated with daily doses of MK-8150 3 mg/kg for 14 days. There were no changes from baseline in these parameters in vehicle-treated animals (data not shown). Values are 18-hour time-weighted average expressed as group mean \pm SEM. * $P < 0.05$, compared to baseline.

In the MD study, there was a 3- to 4-fold accumulation factor at steady state across the dose range tested when a constant dose was administered, which was consistent with MK-8150's PK profile and terminal half-life (terminal half-life ranged from ~46 to 78 hours in the SAD study to ~48 to 79 hours in the MD study).

Clinical Studies

Table 2 summarizes the demographics and baseline characteristics of hypertensive male patients who participated in the SAD study. Table 3 summarizes the demographics and baseline characteristics of 6 panels of hypertensive male patients who participated in the MD study.

Pharmacodynamic Effects of MK-8150 in Hypertensive Subjects

Single doses of MK-8150 up to 90 mg in both healthy and hypertensive subjects resulted in dose-dependent reductions in peripheral BP. Figure 6 shows the mean changes from baseline in pSBP and pDBP over 24 hours following administration of single doses of 5, 24, and 90 mg MK-8150 ($n = 6$ per dose) or placebo ($n = 2$) to hypertensive subjects. The 90-mg dose resulted in a placebo-adjusted least-squares mean \pm SEM change from baseline in pSBP/pDBP of $-16.0 \pm 6.3 / -13.7 \pm 4.0$ mm Hg ($P < 0.05$ for pSBP, $P < 0.01$ for DBP). Across all 3 MK-8150 doses in hypertensive patients, the maximum reduction in SBP and DBP generally occurred 2 to 3 hours following administration of MK-8150. These BP reductions were fairly well preserved until 8 to 16 hours postdose, when they began to decrease in magnitude (Figure 6). There were no significant differences relative to placebo in mean change from baseline in HR weighted over the 24-hour assessment period in any of the 3 MK-8150 doses tested (data not shown).

Ten-day MD clinical studies with MK-8150 were conducted in order to explore the durability of effect. Figure 7 shows the reductions in pSBP and pDBP on day 1 and day 10 after 10 days of once-daily 5, 10, 15, or 20 mg MK-8150 dosing in hypertensive patients. The maximal, placebo-adjusted, TWA_{0-24} mean \pm SE change from baseline in BP on day 1 occurred at the 20 mg dose level and was -11.6 ± 3.5 mm Hg ($P < 0.01$) for pSBP and -14.8 ± 3.9 mm Hg for pDBP ($P < 0.001$). In general, the pSBP changes seen on day 10 appeared to be less than the changes seen on day 1 at a given dose level, although TWA_{0-24} reductions in the 4 to 9 mm Hg range were observed on day 10. Of note is that although the BP-lowering effect appeared less robust on day 10 compared with day 1, PK analyses showed a 3- to 4-fold increase in mean MK-8150 exposure on day 10 compared with day 1 at each dose level. Thus, there was a significant change in the exposure-response relationship over the 10 days of dosing, with increasing

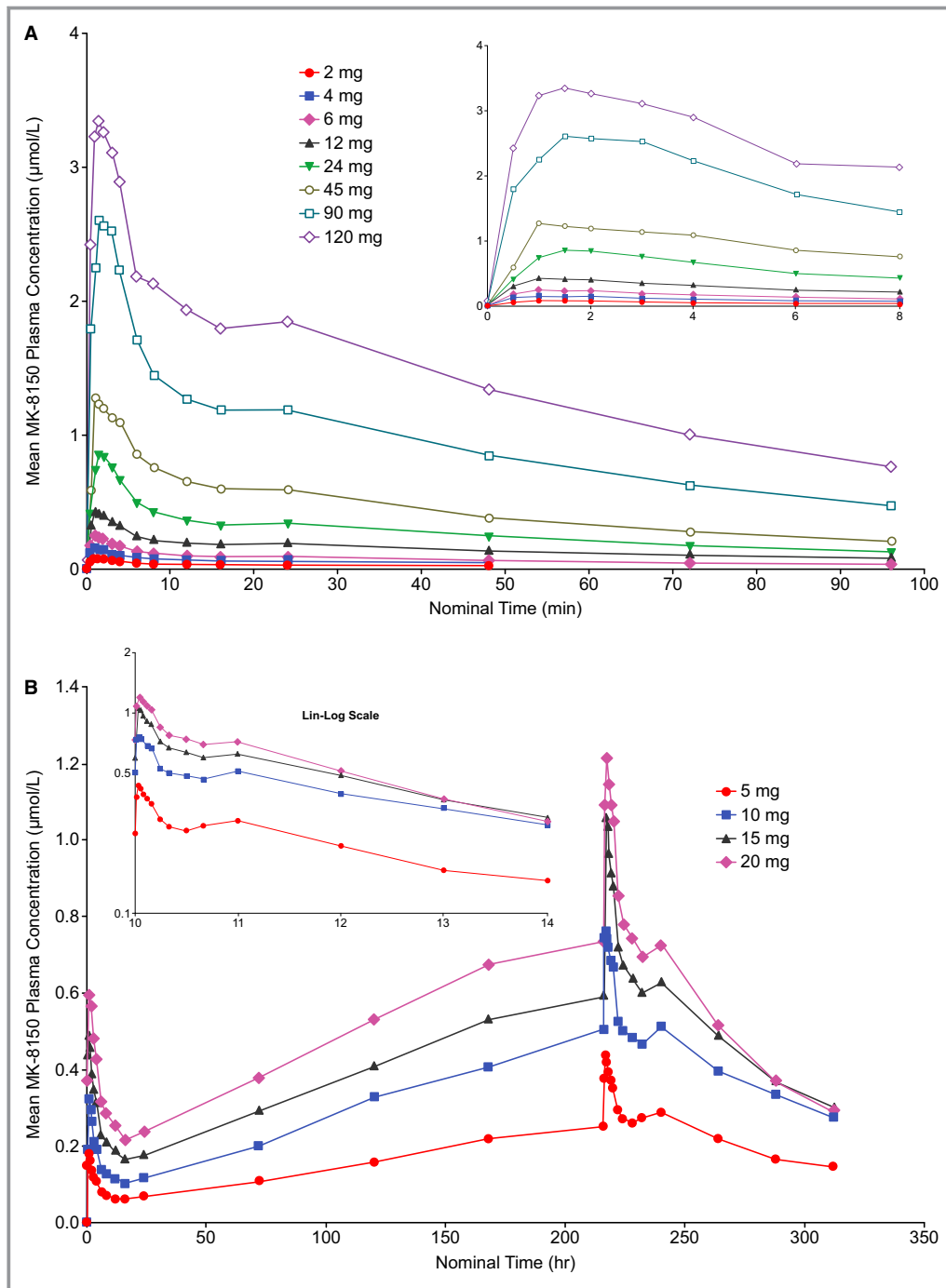


Figure 5. Mean plasma concentration-time plots for MK-8150 in (A) a single-ascending-dose study in healthy male volunteers and hypertensive male patients and (B) a multiple-dose study in hypertensive male patients. A, n=6 for MK-8150 2-, 4-, 6-, 12-, 24-, 45-, and 90-mg groups; n=5 for MK-8150 120-mg group. B, n=6 each for MK-8150 5-, 10-, 15-, and 20-mg groups. HR indicates heart rate.

exposure in the face of decreasing PD effect, raising concern for tolerance.

Based on the shift in PK/PD relationship observed over 10 days in panels 1 to 4, two 28-day studies were then initiated to further investigate the durability of the hemodynamic effects of MK-8150, to assess for tolerance, and to

better understand the change in PK/PD relationship over time. In panel 5, hypertensive subjects received 10 mg MK-8150 daily on days 1 to 7 followed by 20 mg MK-8150 daily on days 8 to 28. The 10-mg dose was selected based on the PK/PD modeling work and was used for the first week in order to minimize adverse events and decrease the chances

Table 2. Demographics and Baseline Characteristics of Male Hypertensive Patients Participating in Panel 3 of the Single-Ascending-Dose Study

Baseline Characteristic	Panel 3 (N=8)
Sex, n (%)	
Male	8 (100)
Mean±SD age, y	51.4±6.8
Race, n (%)	
White	8 (100)
Mean±SD BMI, kg/m ²	51.4±6.8
Mean±SD pSBP, mm Hg	140.4±9.9
Mean±SD pDBP, mm Hg	90.0±7.0
Mean±SD HR, bpm	66.8±8.7

BMI indicates body mass index; HR, heart rate; pDBP, peripheral diastolic blood pressure; pSBP, peripheral systolic blood pressure; SD, standard deviation.

of subjects reaching hemodynamic stopping criteria. Dosing was allowed. Figure 8 shows both the MK-8150 exposure ($\mu\text{mol}/[\text{L}\cdot\text{h}]$) and the placebo-adjusted change in TWA_{0-24} pSBP on days 1, 8, 15, 22, and 28 for panel 9. Although MK-8150 resulted in significant pSBP lowering on days 1 and 8, there was no significant pSBP-lowering effect on day 28 when compared to day 1. The pDBP followed a similar trend, with complete loss of effect compared with placebo by day 28. Further, the exposure was ~ 7.3 -fold higher on day 28 than on day 1, indicating that the loss of MK-8150's BP-

lowering effect occurred in the face of significantly increased exposure to the drug.

Safety and Tolerability of MK-8150 in Humans

Overall, multiple doses of MK-8150 were generally well tolerated in all panels and all populations studied. There were no serious adverse events reported during the treatment period. The most common adverse event was headache. All occurrences of headache were mild or moderate in intensity and resolved spontaneously or with symptomatic medical treatment, except for 1 subject who was receiving MK-8150 20 mg and reported a severe headache and was subsequently discontinued from the study. One subject had a mildly elevated serum creatinine, which was considered related to study drug per investigator assessment. No other clinically significant abnormalities were noted in routine serum chemistries, hematology laboratory assessments, urinalyses, or ECGs in any panel. There were no clinically or statistically significant mean changes in the TWA_{0-24} HR at any MK-8150 dose tested in any panel.

Discussion

This article reports the results of a hypothesis-driven translational research effort to create a next-generation oral NO donor with no induction of tolerance to its hemodynamic effects in humans and with improved efficacy, safety, and tolerability due to more favorable PK properties. The key

Table 3. Demographics and Baseline Characteristics of Male Hypertensive Patients Participating in Panels 1 to 4, 9, and 10 of the Multiple-Dose Study

Baseline Characteristic	Panel 1 (N=8)	Panel 2 (N=8)	Panel 3 (N=8)	Panel 4 (N=7)	Panel 9 (N=18)	Panel 10 (N=18)
Sex, n (%)						
Male	8 (100.0)	8 (100.0)	8 (100.0)	7 (100.0)	18 (100.0)	18 (100.0)
Mean±SD age, y	46.1±6.3	41.3±10.8	42.0±11.4	42.7±10.8	51.5±8.5	54.8±9.6
Race, n (%)						
Asian	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)	0 (0.0)
Multiple	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
White	7 (87.5)	8 (100.0)	8 (100.0)	7 (100.0)	17 (94.4)	18 (100.0)
Ethnicity, n (%)						
Hispanic or Latino	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Not Hispanic or Latino	8 (100.0)	7 (87.5)	8 (100.0)	7 (100.0)	18 (100.0)	18 (100.0)
Mean±SD BMI, kg/m ²	28±2	29±3	27±4	27±2	27±2	28±2
Mean±SD pSBP, mm Hg	150±12	137±7	142±17	139±5	145±9	147±10
Mean±SD pDBP, mm Hg	92±6	86±9	90±10	86±7	89±6	90±10
Mean±SD HR, bpm	65±14	63±7	66±12	61±10	62±10	66±9

BMI indicates body mass index; HR, heart rate; pDBP, peripheral diastolic blood pressure; pSBP, peripheral systolic blood pressure; SD, standard deviation.

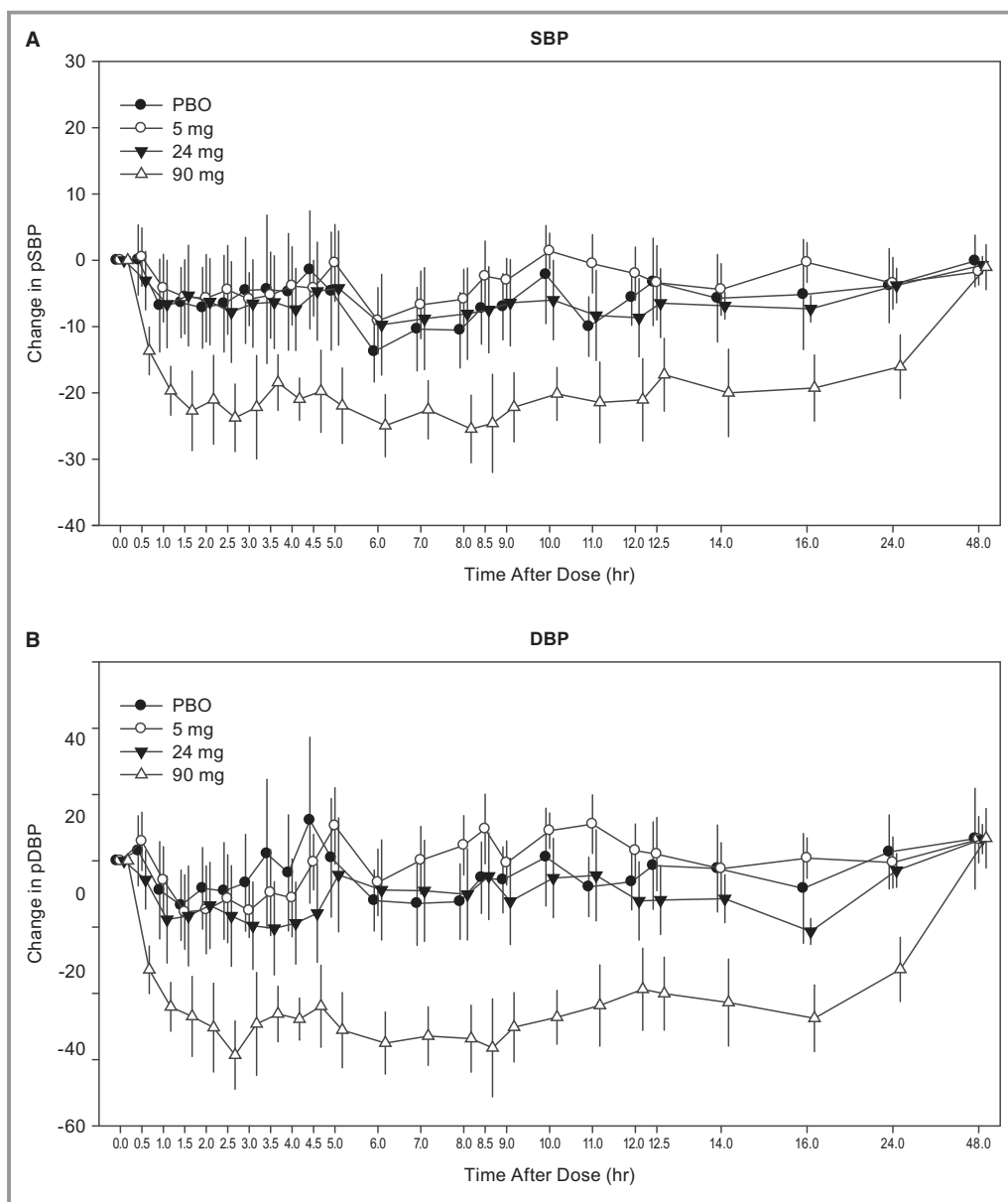


Figure 6. Mean±SE change from baseline in (A) peripheral systolic blood pressure (pSBP; mm Hg) and (B) peripheral diastolic blood pressure (pDBP; mm Hg) over 24 hours following single-dose administration of placebo (PBO) and MK-8150 5, 24, and 90 mg in hypertensive male patients. n=6 for PBO and MK-8150 90-mg groups; n=5 for MK-8150 5- and 24-mg groups.

pharmacologic differentiator for MK-8150 is its unique biochemistry of NO release through metabolism by CYP3A4, which, in vitro, allows for comparatively slow and steady release of NO. We hypothesized that this unique metabolic mechanism of NO generation would (1) be less prone to induce tolerance in humans because the biochemistry of NO generation and metabolism from organic nitrate NO donors is thought to be a contributor to the mechanism of nitrate tolerance, and (2) improve safety and tolerability in humans by delivering a consistent concentration of NO over time with a slow initial onset and reduced peak:trough ratio. In vitro liver

preparation studies using analysis of concurrently formed metabolites across species (rat, dog, and human) convincingly demonstrated the production of NO from MK-8150 through a CYP3A4-mediated metabolic step. The slow rate of this metabolic step resulted in a sluggish production of NO in vitro, supporting the desired target profile of the drug. In vitro evidence of NO release was corroborated by demonstration of increased cGMP levels with Compound 2, a close diazeniumdiolate analogue of MK-8150.

In the dog, a single 3 mg/kg dose of MK-8150 lowered SBP by ~20 mm Hg. Peak BP-lowering response was reached

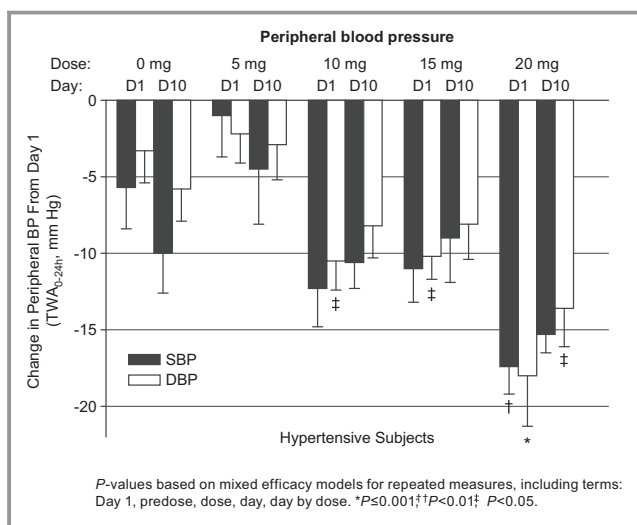


Figure 7. Mean±SE peripheral systolic (pSBP) and diastolic (pDBP) blood pressure (time-weighted average [TWA]_{0-24h}, mm Hg) at day 1 and day 10 for MK-8150 5, 10, 15, and 20 mg in hypertensive male patients participating in a 10-day multiple-dose study. n=2 for placebo (0 mg) group; n=6 each for MK-8150 5-, 10-, 15-, and 20-mg groups. **P*<0.05, compared to placebo.

at ~2 hours post treatment and lasted up to 18 to 20 hours. A desirable peak:trough BP-lowering ratio of <2 was achieved. In contrast, peak BP-lowering response to ISMN occurred within 1 hour, and the effect lasted ~12 hours (data not shown). The relatively slow onset of BP lowering and longer-

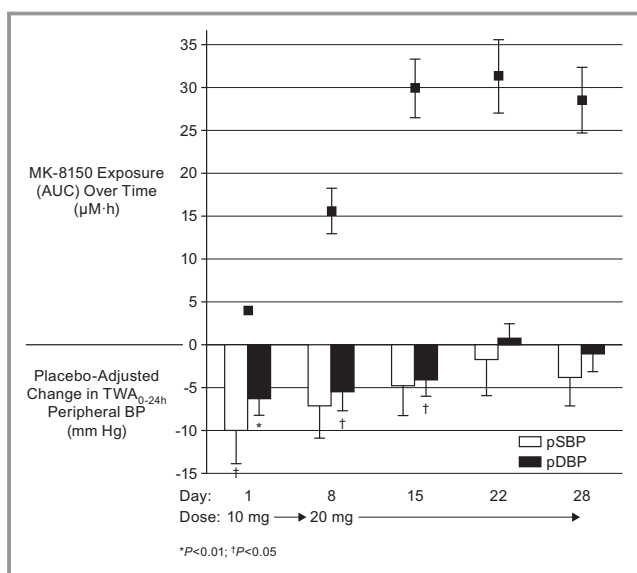


Figure 8. Mean±SE MK-8150 exposure (AUC₀₋₂₄ [µmol/L×hour]) and placebo-adjusted change from baseline in peripheral systolic (pSBP) and diastolic (pDBP) blood pressure over time in hypertensive male patients participating in Panel 9 of the 28-day multiple-dose study. n=12 patients on MK-8150 10 mg QD on Days 1 to 7 followed by MK-8150 20 mg QD on Days 8 to 28; n=6 patients on placebo. **P*<0.05, compared to placebo.

lasting effect of MK-8150 confirmed the NO release kinetics observed in vitro and presented a favorable in vivo PD profile in animals. In the 14-day study, a similarly robust BP-lowering response was observed for the full duration of the study, which suggested that MK-8150 may be able to overcome tolerance. However, the half-life of MK-8150 in dogs was 19 hours, as opposed to it being up to ~79 hours in humans from the MD study findings.

Based on its encouraging overall preclinical profile, MK-8150 was advanced into the clinic. Nitrates have multiple physiologic cardiovascular effects in humans, and we focused on peripheral BP as a biomarker for NO generation and pharmacologic activity in our early clinical studies. The SAD study showed robust BP-lowering efficacy in both healthy (data not shown) and hypertensive patients, although the onset was somewhat faster than (T_{max}=1-2 hours), and the resulting PD and tolerability were somewhat different from those predicted by preclinical studies. The reductions in pSBP and pDBP at T_{max} approached 30 mm Hg. Headaches were common at high doses, although they were generally mild or moderate in intensity and usually resolved spontaneously without treatment. Importantly, the half-life of MK-8150 was found to range from ~46 to 78 hours, suggesting that once-daily dosing would be feasible, although it did raise some concerns about the accumulation ratio of MK-8150 and what this would mean clinically with regard to PD effects. Nonetheless, we were eager to determine whether the hemodynamic effects would be sustained in MD studies, as was observed in our telemetered dog experiments.

Because tolerance to nitrovasodilators generally begins to develop after 24 to 48 hours of continuous dosing, we initially conducted a 10-day MD study with MK-8150 in hypertensive patients. Our results showed significant BP lowering with MK-8150 on day 1, but this effect was not fully maintained after 10 days of daily dosing. As the concentration of MK-8150 was found to increase 2.8- to 3.7-fold on day 10 compared with day 1, the compound was well tolerated, but the diminishing PK/PD response was concerning, as it indicated the potential development of tolerance.

In order to investigate the apparent erosion of the PK/PD relationship over time in the 10-day studies and to further evaluate the durability of MK-8150's hemodynamic effects, a 28-day MD study was undertaken, and full 24-hour BP monitoring was performed once weekly on days 1, 8, 15, 22, and 28. Despite potent BP lowering on day 1, all BP-lowering effects of MK-8150 relative to placebo were lost by day 28, and this occurred despite an ~7-fold increase in MK-8150 concentration on day 28 relative to day 1. Thus, the apparent uncoupling of PK from PD seen on day 10 in the 10-day study continued, such that there was a complete loss of BP-lowering response after 28 days of daily MK-8150. The tolerability profile of MK-8150 improved over time, with the incidence of

headache, which is generally thought to be secondary to NO exposure,¹³ ultimately decreasing to 0% on day 28 across all studies. A second 28-day study in 18 hypertensive subjects testing higher doses up to 40 mg MK-8150 daily (Panel 10) also showed a complete loss of BP-lowering effect on day 28 compared to day 1 (data not shown).

These results raise many questions regarding the investigation and development of novel NO donors and the mechanism of tolerance to these active molecules. First, the durable effects of MK-8150 in the 14-day dog study, which did not reveal evidence of tolerance, did not translate to humans. However, it is certainly possible that the dogs would have developed tolerance to MK-8150 if the treatment period had been extended to 28 days, as observed in humans. Further, there was significant compound accumulation on chronic administration of MK-8150 in humans (3- to 4-fold after 10 days and up to 7-fold after 28 days), while concentrations of MK-8150 and its metabolites were almost identical on day 1 and day 14 in the dog (0.436 $\mu\text{mol/L}$ on day 1 vs 0.456 $\mu\text{mol/L}$ on day 14). It is not known whether accumulation of MK-8150 in humans was a contributory factor to the observance of tolerance.

The explanation for the loss of BP lowering over time with MK-8150 in humans is likely multifactorial. Chronic pharmacologic BP lowering with NO donors generally leads to neurohormonal activation, vasoconstriction, and volume expansion, a phenomenon dubbed pseudotolerance in the literature,⁶ and there was almost certainly an element of this at play in our MD clinical studies. Other potential contributors are altered metabolism of MK-8150 over time, leading to a decrease or loss of NO generation by day 28, and increased NO clearance over time. Munzel and others have extensively studied the role of reactive oxygen species as NO scavengers, and tolerant blood vessels have been shown to have higher levels of reactive oxygen species than normal vessels.^{14,15} This is thought to occur because organic nitrates induce oxidative stress in the vasculature.

Our results demonstrate that despite robust BP lowering during the first 1 to 2 weeks of MK-8150 administration, the effects ultimately waned over 28 days of daily dosing in humans. These findings reinforce the prevailing view that the development of tolerance to NO donors is a complicated and likely multifactorial process and demonstrate that utilizing a novel mechanism of NO release through CYP3A4 metabolism was ultimately insufficient to overcome the development of tolerance to the BP-lowering effects of this novel NO donor.

Author Contributions

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Mitra, Sun, Lo, Cully, and A. Ali are responsible for the work described in this paper. All authors were involved in at least 1 of the following: conception, design, acquisition, analysis, statistical analysis, and interpretation of data in addition to drafting the manuscript and/or revising/reviewing the manuscript for important intellectual content. All authors provided final approval of the version to be published. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Disclosures

Knox, de Kam, Azer, Wong, Ederveen, Shevell, Morabito, Meehan, Liu, Reynders, Deneff, Mitselos, Jonathan, Gutstein, Mitra, Sun, Lo, Cully and Ali are current or past employees of Merck Sharp and Dohme Corp, a subsidiary of Merck & Co, Inc, Kenilworth, NJ, and may own stock and/or hold stock options in the Company.

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