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Comparison of Telemetry and High-Definition Oscillometry for Blood Pressure Measurements in Conscious Dogs: Effects of Torcetrapib

Olivier Meyer, Roland Jenni, Andrea Greiter-Wilke, Alexander Breidenbach, and Henry H Holzgrefe*

This study compared torcetrapib-induced blood pressure (BP) changes simultaneously obtained by high-definition oscillometry (HDO) and telemetry. Male beagles (n = 6) received single oral doses of vehicle or torcetrapib at 10 or 30 mg/kg; BP were acquired simultaneously by HDO and telemetry from 2 h before dosage until 7 h afterward. Systolic, diastolic, and mean arterial pressures (MAP) and heart rate were compared by using Altman-Bland agreement analysis. Dogs were allocated into subgroups according to temperament and baseline MAP (less than 110 mm Hg and 110 mm Hg or greater). Both methods demonstrated high precision. HDO recordings exhibited higher variability for all parameters (inclusive MAP SDs were 7.0 ± 2.7 mm Hg for HDO compared with 3.4 ± 1.9 mm Hg for telemetry), accompanied by a positive bias for all pressures (systolic, 10.4 mm Hg; diastolic, 5.7 mm Hg; MAP, 1.9 mm Hg). Both methods detected similar maximal increases in MAP with 30 mg/kg torcetrapib (HDO, 15.8 ± 10.4 mm Hg; telemetry, 15.8 ± 5.3 mm Hg). No significant effects were noted for heart rate. Torcetrapib elicited a dose-dependent increase in BP in dogs with baseline MAP of less than 110 mm Hg, whereas increases were maximal with 10 mg/kg in the other group, and dose-dependence was no longer observed. BP changes were influenced by animal temperament, demonstrating that HDO results must be interpreted with caution. HDO may provide a useful and accurate method for noninvasive BP measurements in canine studies.

Abbreviations: AUC, area under the time–concentration curve; BP, blood pressure; HDO, high-definition oscillometry; MAP, mean arterial pressure.

The beagle dog is a common animal model for cardiovascular safety and toxicology studies due to the cardiovascular similarities between dogs and humans. As such, dogs are an accepted predictor for the pharmacologic effects of drugs in humans and have been recognized by regulatory authorities as suitable for pharmacodynamic studies, including telemetry studies. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) regulatory guidelines state that safety pharmacology core battery studies must investigate the effects of the test substance on vital organs and systems including the cardiovascular, respiratory, and central nervous systems.[11,12] To assess possible effects on the cardiovascular system, blood pressure (BP), heart rate, and electrocardiogram should be evaluated. Monitoring arterial BP in conscious dogs requires a precise, accurate, and well-accepted measurement system. In a repeated-measures experimental design, precision (reproducibility) is more important than absolute accuracy because it is reproducibility that enables the detection of a possible drug effect.[7] Currently, only externally calibrated invasive techniques such as indwelling arterial catheters provide the requisite precision for accurate and reproducible BP assessment.

Telemetry is accepted as the reference method for safety pharmacology studies in large animals because it allows for undisturbed acquisition of several physiologic parameters and is very well tolerated. But telemetry's high cost and absolute requirement for invasive surgical instrumentation greatly limits its use in chronic toxicology studies, which primarily are conducted as terminal studies in large numbers of animals. As a consequence, a noninvasive method for BP measurement that fulfills all previous requirements and is applicable for routine and repeated use could greatly facilitate hemodynamic data acquisition in the absence of invasive measurements. Ideally, such a system would function in real time and be able to discriminate between waveforms that are associated with presystolic amplitudes, increasing amplitudes (that is, systolic pressure), mean arterial pressure (MAP), decreasing amplitudes (that is, diastolic pressure), and artifacts.[4]

High-definition oscillometry (HDO; S and B MedVet, Babenhausen, Germany) enables interactive, real-time evaluation of each BP measurement by using a computer-generated display of BP waveform amplitude scans. Previous oscillometric BP technologies directly measured only MAP and derived systolic and diastolic pressures by using a computer algorithm, a process that can result in biased measurements under several hemodynamic conditions.[18] In contrast, the currently state-of-the-art HDO methodologies are unique in that that they accomplish direct measurement of systolic, diastolic, and mean arterial pressures by means of proprietary pressure waveform analyses.

The purpose of the current study was to compare peripheral arterial BP obtained by using HDO technology with simultaneous invasive telemetric measurements of central aortic pressure. To mimic a typical safety pharmacology study environment, periodic HDO pressure measurements were performed in dogs (n = 6) before and after administration of 10 or 30 mg/kg doses of torcetrapib, an agent that previously has been shown to significantly increase BP in dogs.[5,20] The halt of torcetrapib

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F Hoffmann-La Roche, Pharmaceuticals Division Non-Clinical Drug Safety, Basel, Switzerland.
Corresponding author. Email:henryholzgrefe@hotmail.com

development after the ILLUMINATE study raised questions regarding the preclinical detection of torcetrapib-induced increases in BP.[5,10,24] Accordingly, we compared time-matched HDO measurements with simultaneously obtained telemetric values to assess the reliability of HDO technology in the context of a typical safety pharmacology study.

Material and Methods

Drug preparation and administration. Torcetrapib powder was synthesized (F Hoffman-La Roche, Basel, Switzerland) as the free base and administered by oral gavage. The vehicle used was soybean oil, which was selected in light of the documented solubility of torcetrapib in olive oil.[19] Internal tests confirmed that the solubility of torcetrapib in soybean oil was 40 mg/mL. Fresh solutions were prepared daily and administered at dose levels of 10 and 30 mg/kg in a constant dose-volume of 5 mL/kg. Each dog received a single dose of vehicle on day 1 and torcetrapib on days 4 (30 mg/kg) and 11 (10 mg/kg), resulting in a washout period of 7 d between active doses. Individual doses were based on the most recent body weights. Doses were selected according to unpublished internal exposure data obtained in cynomolgus monkeys and dogs; torcetrapib demonstrated a reproducible BP increase of 7 to 20 mm Hg in both species after oral administration of 30 mg/kg.

Animals. All animals used in this study were cared for according to the *Guide for the Care and Use of Laboratory Animals*.[13] The experimental procedures used in the present investigation received prior approval from the City of Basel Cantonal Animal Protection Committee and were performed in accordance with all applicable international, federal, and local regulations. The testing facility was fully AAALAC-accredited.

Purpose-bred male beagle dogs (n = 6; age, 4 to 5 y) were used in this study. All dogs were instrumented with telemetry transmitters (Konigsberg Implant T27F-11B, Integrated Telemetry Services, Pinckney, MI) between November 2004 and November 2005. Dogs weighed between 9.55 and 14.90 kg (mean ± 1 SD, 11.8 ± 1.9 kg) and were weighed on the morning of each dosing day. Dogs were housed in groups of 3 throughout the study period, with the exception of dosing days, when they were housed individually. Daily, all dogs were allowed to have a free-exercise run with their cohorts for approximately 45 min. Room environmental conditions were controlled with respect to temperature (18 °C ± 2 °C) and humidity (40% to 80%) with an alternating 12:12-h light:dark cycle. Music from a local radio station was played during light hours. Tap water was provided ad libitum. Food (catalog no. 3358, Provimi, Kliba, Kaiseraugst, Switzerland) was offered daily at 1100 on nondosing days and at 3 h after treatment on dosing days, after a scheduled pharmacokinetic blood sampling. Dogs were dosed between 1100 and 1200 and were observed for adverse effects at 2, 3, 5, and 7 h after dosing.

Telemetry implantation. All procedures were conducted according to standard aseptic surgical techniques. Dogs were fasted overnight before surgery. Atropine (0.1%; 1 mL SC) was given as premedication, and anesthesia was induced by propofol (5 to 8 mg/kg IV) followed by inhalation of isoflurane to maintain a surgical plane of anesthesia. Postsurgical analgesia was provided by administration of buprenorphine (0.01 to 0.02 mg/kg) for at least 5 d. Prophylactic antibiotics (amoxicillin and clavulanic acid 10 mg/kg twice daily) were administered for at least 7 d. The telemetric transmitter and battery module were implanted between the external and internal abdominal muscle layers in the left flank. A left thoracotomy was performed at the fifth intercostal space, and a Konigsberg pressure transducer was placed in the descending aorta just below the aortic arch. This transducer also served as the positive electrode for lead II electrocardiography. The reference electrode was anchored subcutaneously near the apex of the heart. A left ventricular pressure transducer was positioned in the left ventricle through the apex of the heart. All surgical procedures were performed in accordance with the manufacturer's recommendations.

HDO. BP measurements by HDO (Memo Diagnostic 1.36.02 hardware, S and B MedVet, Babenhausen, Germany) used a type C2 cuff. Primary analysis was performed by using MDS software (version 1.7.5.2; S and B MedVet).

HDO data acquisition. During the week prior to the study, 2 acclimating HDO simulations were performed in all subjects. Dogs were conscious and manually restrained in a standing position and the HDO cuff fixed at the base of the tail. It has been widely documented that coccygeal artery at the base of the tail is the most suitable cuff site for reliable BP measurements in dogs.[2, 21] Two persons including the examiner were needed to restrain the animal in such a way that movement artifacts were minimized to the extent possible to reduce or eliminate noise in the HDO signal. To this end, the first person restrained the dog to minimize general movement, while the second person (the examiner) restrained the tail and hindquarters. After the cuff was applied at the base of the tail, the measurements commenced. With the current HDO equipment, measurement of the circumference of the cuff site was not necessary, because the HDO device detected the cuff volume and derived information on arterial diameter during the first reading and then automatically implemented the relevant parameter adjustments. The HDO device recorded systolic, diastolic, MAP, and pulse rate. The current study consisted of 3 dosing days where successive doses of 0, 30, and 10 mg/kg torcetrapib were administered by oral gavage. Data were acquired from all animals at 2 and 1 h before dosing as well as at 2, 5, and 7 h after dosing. A minimum of 6 recordings were performed for each scheduled HDO time point, depending on the quality of the individual measurements. Quality verification was performed and recordings exhibiting excessively high amplitudes, atypical HDO signals, or significant artifacts were considered technically inadequate and were discarded. During periods between HDO acquisitions, all dogs were undisturbed and allowed to rest quietly.

Telemetry acquisition. Aortic and left ventricular pressure signals as well as an approximate lead II electrocardiogram were collected (CA Recorder hardware and software systems, RMISS, Wilmington, DE, and DISS, Pinckney, MI). Physiologic signals were digitized at sampling rates of 250 Hz (BP) or 500 Hz (electrocardiogram) and transmitted to an associated base station receiver (RMISS). Implants for each dog used discrete transmitter frequencies to eliminate crosstalk between subjects. The receiver converted the transmitted signals to analog voltages, which were analyzed by CA Recorder software (version 2.2.3, RMISS). Cardiovascular signals were acquired continuously and expressed as beat-to-beat values. Telemetric data acquisition commenced at least 2.2 h before dosing and continued for 18 h after dosing. Systolic, diastolic, MAP, and heart rate were derived from the aortic pressure and electrocardiogram waveforms and were continuously recorded during HDO measurements. After data acquisition, the entire individual dataset was replayed to verify fiduciary marks. Data then were imported into a custom automated spreadsheet (Excel, Microsoft, Redmond, WA) in which physiologic filters were applied to remove environmental and electrical noise.

With traditional BP measurements as well as with telemetry, MAP is derived from systolic and diastolic values. In traditional

oscillometry, MAP is calculated as the diastolic pressure + 1/3 of the pulse pressure; systolic and diastolic pressures are measured directly. The current telemetry analysis software (CA Recorder, RMISS) derives MAP according the expression

$$\sum_{i=1}^{N} X_i / N$$

where X is the sample value and N is the number of samples in the cycle of the aortic pressure. In contrast, HDO provides a direct measurement of MAP defined as the mean of the BP values during the plateau between ascending systolic pressure amplitudes and the descending diastolic amplitudes. Agreement analysis between HDO and telemetry pressure measurements demonstrated that MAP was associated with the least bias. Accordingly, we selected MAP as the primary comparator between the 2 BP measurement techniques. The use of MAP as the principle comparator is consistent with previous studies, which have demonstrated that MAP provides the best agreement between direct BP measurement and oscillometry.[6,16,25]

Because chronically instrumented telemetry devices are characterized by variable baseline drift,[3,23] all pressure transducers in the current experiment were adjusted daily to a reference zero (baseline) value as recommended by the manufacturer. Briefly, the instantaneous left ventricular minimal pressure was assumed to be 0 mm Hg. By using the unique manufacturer-supplied gain setting, each transducer was adjusted as needed to reset the left ventricular minimum pressure to 0 mm Hg. The aortic systolic pressure then was matched to the resulting left ventricular systolic pressure. This procedure assumes that transducer gain remains constant over time and that there is no amplitude drift. In the event that the left ventricular transducer became unusable, the aortic pressure was adjusted for possible drift by matching the systolic pressure to a historic mean value for that subject, with both measurements obtained at a common heart rate. The accuracy and reproducibility of these pressure adjustment techniques were assessed by comparing the pressures and heart rates obtained yearly over the history of each subject with those values obtained during the current study. To characterize transducer stability, systemic BP (drift), pulse pressures (gain), and heart rate were obtained after implantation and yearly thereafter from historic data for each subject and compared with the pretest values obtained in the current study.

Statistical methods. For comparative analysis, HDO measurements were time-matched precisely to the telemetry recordings, essentially providing a direct beat-to-beat comparison for all data. Reproducibility (precision) was assessed as the mean SD for all repeated measurements for each scheduled time point by subject and treatment. To adjust for possible handling effects, all data were expressed initially as the change from the individual predose baseline and then presented as the time-matched change from the respective individual vehicle treatment. Agreement between HDO and telemetric measurements was assessed by Altman–Bland analysis.[1] Changes in pressure also were expressed as the area under the time–concentration curve from 0 to 7 h ($AUC_{0\rightarrow7}$), as estimated by the trapezoidal rule[8] and compared by using one-way ANOVA followed by least-squares difference posthoc contrast to identify individual significant changes. All statistical procedures were performed by using Analyze-It software (Analyze-it Software, version 2.21, Leeds, UK). In all cases, a P value of 0.05 or less was considered statistically significant. Because all data demonstrated normality according to the Shapiro-Wilk method, data were expressed as mean ± 1 SD where appropriate. To broadly characterize the possible effects of subject temperament on treatment effects, dogs were judged subjectively as either calm (group I) or nervous and excited (group 2) prior to dosing. For analysis, dogs were objectively subdivided into 2 groups according to baseline MAP (group I [n = 4], MAP < 110 mm Hg; group 2 [n = 2], MAP ≥ 110 mm Hg). MAP baseline values were defined as the average of telemetric MAP from 2.2 to 0.1 h before torcetrapib treatment on each dosing day. Subjective assessment of behavior was performed by the examiner.

Results

Stability of implanted telemetry pressure transducers. Mean aortic systolic and pulse pressures were collected for 24 h from male beagle dogs approximately 1 mo and 1 y after implantation and 6 to 8 mo before entry into the current experiment; representative summary data for each subject are presented in Table 1 (systolic; drift) and Table 2 (pulse pressure; gain). In all cases, pressures remained stable both within the current experiment and over time. Compared with the 1-y post implantation values, no significant differences were noted in current data for any subject. Over the same period, mean diastolic pressures demonstrated similar stability, varying from 73.3 ± 10.4 to 79.9 ± 11.8 mm Hg and heart rate ranged from 78.3 ± 4.5 to 94.0 ± 10.2 bpm.

Torcetrapib exposure. Toxicokinetic data confirmed that all dogs were exposed at each dose but that exposures were not dose-proportional. At 3 h after dose, mean torcetrapib plasma concentrations were less than 10, 1462, and 918 ng/mL for 0 (vehicle only), 10, and 30 mg/kg respectively. Emesis was observed 2 h after dose in 1 of 6 dogs after the 10 mg/kg and in 3 of 6 dogs after the 30 mg/kg dose.

Repeatability and reproducibility. Obtaining 6 to 14 (mean ± 1SD, 9 ± 1.6) HDO measurements per scheduled time point on conscious and manually restrained dogs required approximately 6 min (range, 3 to 11 min), depending on the behavior of the subject. From all completed measurements (n = 810), 15% were discarded after a quality verification whereas the remaining 85% were deemed technically adequate. Subsequent to quality verification, a mean of 7.7 ± 1.6 technically adequate measurements were obtained for each scheduled HDO time point for each animal. The mean standard deviation for repeated HDO MAP measurements for each scheduled time point was 7.0 ± 2.7 mm Hg whereas that for simultaneous telemetric MAP measurements was 3.4 ± 1.9 mm Hg (Table 3), demonstrating that although both techniques were highly reproducible, telemetric measurements demonstrated greater stability over common measurement intervals.

Agreement analysis. The mean difference between simultaneous MAP measurements (n = 686) by HDO and telemetry was –0.6 ± 14.7 mm Hg, with telemetry values tending to be slightly lower. According to the Association for the Advancement of Medical Instrumentation scheme for comparison of BP measurements,[15] method differences were less than 5, 10, and 15 mm Hg for 25%, 46%, and 66% of all measurements, respectively. Altman–Bland agreement analysis between HDO and telemetric measurements for vehicle-treated animals demonstrated significant (P < 0.01) HDO biases of 10.4 and 5.7 mm Hg for systolic (Figure 1) and diastolic (data not shown) pressures, respectively. The bias for MAP was 1.9 mm Hg and did not achieve significance. The associated 95% confidence intervals were –21.4 to 43.8, –26.8 to 39.6, and –27.5 to 33.0 mm Hg for systolic, diastolic, and mean arterial pressure, respectively. Heart rate exhibited no significant bias between methods, for which the 95% confidence intervals was –11.6 to 4.9 bpm. The

Table 1. Stability over time of systolic pressures measured telemetrically

Dog	1 mo after implantation	1 y after implantation	6 to 8 mo before entry into current study	Current pretest value 1	Current pretest value 2
1	131	129	128	124	129
2	126	129	133	118	116
3	113	132	124	121	128
4	121	111	113	107	110
5	144	104	104	103	116
6	148	124	113	136	138
Mean	131	121	119	118	123
1 SD	13.2	11.4	10.8	12.1	10.5

Mean systolic pressures were collected for 24 h. Current pretest values were separated by 11 d. Systolic pressures decreased slightly at 1 y postimplantation and remained stable thereafter. Importantly, the very stable systolic pressures confirm effective control of possible baseline drift over time.

Table 2. Stability over time of pulse pressures measured telemetrically

Dog	1 mo after implantation	1 year after implantation	6 to 8 mo before entry into current study	Current pretest value 1	Current pretest value 2
1	49	47	48	48	53
2	51	49	46	50	49
3	53	53	52	47	48
4	42	36	35	29	31
5	48	39	44	39	46
6	60	53	50	51	51
Mean	51	46	46	44	46
1 SD	6.1	7.1	6.0	8.7	7.9

Mean pulse pressures were collected for 24 h. Current pretest values were separated by 11 d. Similar to systolic pressures, pulse pressures decreased slightly at 1 y postimplantation and remained stable thereafter. The very stable pulse pressures confirm the transducer gain remained constant over time.

Table 3. Variability of simultaneous hemodynamic measurements (mean ± 1 SD) by method

		Torcetrapib dose (mg/kg)		
		0	10	30
HDO				
	MAP (mm Hg)	7.6 ± 2.9	7.1 ± 2.7	6.2 ± 2.4
	Systolic pressure (mm Hg)	8.5 ± 3.6	9.9 ± 4.8	8.7 ± 3.4
	Diastolic pressure (mm Hg)	8.4 ± 3	7.1 ± 2.4	6.8 ± 2.7
	Heart rate (bpm)	6.8 ± 3.2	7.2 ± 2.7	7.5 ± 3.5
Telemetry				
	MAP (mm Hg)	3.8 ± 1.9	2.7 ± 1.7	3.7 ± 1.9
	Systolic pressure (mm Hg)	4.9 ± 2.8	3.5 ± 2.3	4.8 ± 2.6
	Diastolic pressure (mm Hg)	3.4 ± 1.6	2.5 ± 1.4	3.2 ± 1.5
	Heart rate (bpm)	4.7 ± 2.4	4.3 ± 1.8	6.1 ± 4.9

Standard deviation (mean ± 1 SD) for repeated measurements for all scheduled time points are presented for each hemodynamic parameter by method. Both methods demonstrated high intraindividual precision for all parameters, although HDO exhibited slightly greater standard deviations for all parameters.

Altman–Bland correlation was 0.96 for heart rate (Figure 2) and less than 0.2 for the other hemodynamic variables.

Effects of torcetrapib. Both telemetry and HDO detected approximately equivalent treatment-related increases in systolic, diastolic, and mean arterial BPs commencing approximately 2 h after dose whereas no significant changes in heart rate were noted with either method. With HDO (Figure 3 A), normalized increases in systolic, diastolic, and mean arterial pressure were detected at 2 and 5 h after dose. At 7 h after dose, the HDO pressures were decreased compared with telemetric measurements but were still elevated compared with those from vehicle only. Of note, evidence for a dose-dependent effect was no longer observed at 7 h. In contrast, normalized telemetric data (Figure 3 B) demonstrated sustained increases in systolic, diastolic, and mean arterial pressure from 2 to 7 h after dose. For comparison, continuous raw (Figure 4) and normalized (Figure 5) telemetric data are presented. From 2 to 7 h, torcetrapib at 10 and 30 mg/kg induced inclusive mean telemetric MAP increases of 11.8 ±

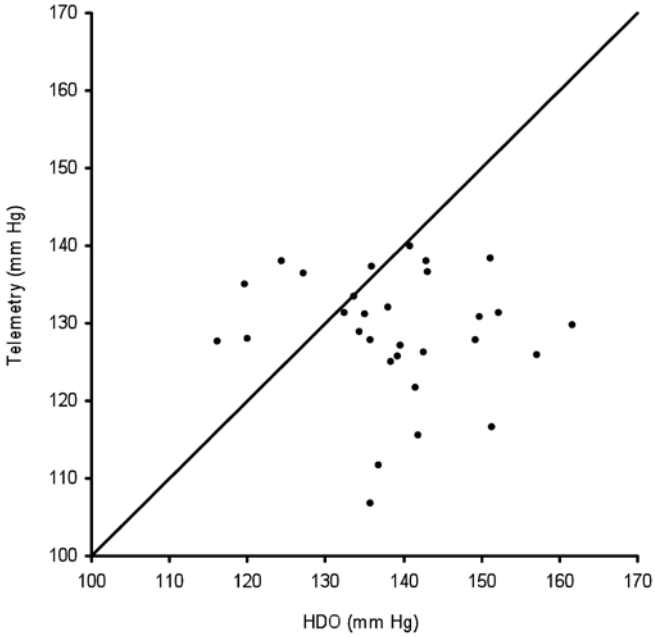

Figure 1. Altman–Bland analysis of systolic pressures obtained by using HDO and telemetry demonstrated a significant ($P = 0.0004$) HDO bias of 10.4 mm Hg. A similar bias was noted for diastolic pressure (data not shown). MAP values were virtually identical (bias, 1.9 mm Hg) between methods and thus were chosen for method comparison.

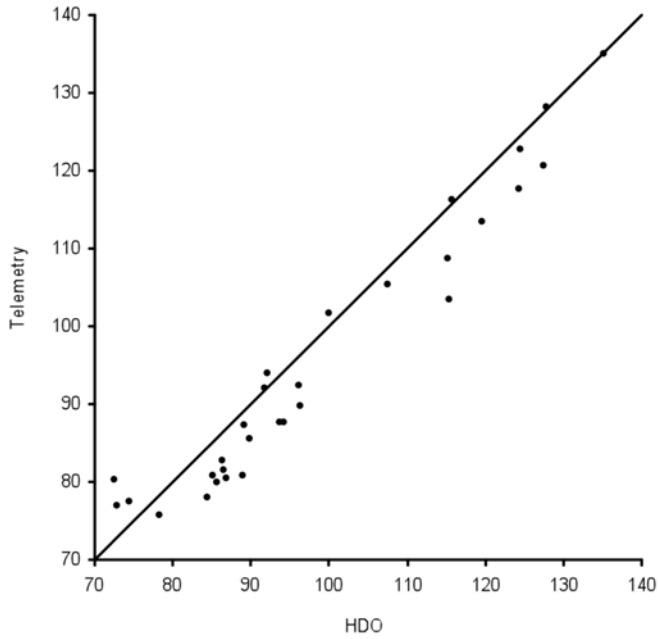

Figure 2. Altman–Bland analysis of heart rates derived from the pulse pressures obtained by using HDO and telemetry demonstrated close correlation between methods. The HDO bias was 3.4 bpm and did not achieve significance.

7.1 and 15.8 ± 5.3 mm Hg compared with temporally matched HDO values of 9.0 ± 11.9 and 15.8 ± 10.4 mm Hg, respectively. For MAP, the telemetric AUC $_{0\rightarrow7}$ were 973, 1071, and 1123 mm Hg·h for the 0, 10, and 30 mg/kg doses, respectively. These pressures increased in a linear and dose-dependent manner ($r^2 = 0.87$). Similar dose-dependent changes in $AUC_{0\rightarrow7}$ were noted for systolic and diastolic pressures ($r^2 \geq 0.94$ for each parameter). Because exposures were similar in all animals, emesis was unlikely to have affected the group responses.

Effects of handling and behavior. When dogs were grouped according to their temperament and baseline MAP (Table 3), dose-dependent increases in MAP were noted with torcetrapib in group 1, for which baseline MAP was less than 110 mm Hg. In contrast, in group 2 (baseline MAP equal to 110 mm Hg or greater), dose-dependent increases in BP with torcetrapib were no longer evident (Figure 6). By protocol design, dogs were undisturbed for 3 scheduled 1-h intervals between HDO measurements. During these 1-h intervals, the dogs progressively relaxed and, after 50 min, demonstrated apparent full recovery from any handling-induced hemodynamic changes. Three 10-min intervals of telemetric data simultaneously acquired during the performance of HDO measurements obtained at –2, –1, 2, 5, and 7 h were compared with the first undisturbed 10-min interval after the vehicle dose. The individual mean difference in HDO-determined MAP between handling-associated and the predefined undisturbed time points was 9.8 ± 4 mm Hg in group 1 dogs and 34.9 ± 4.5 in group 2 dogs ($P < 0.05$).

Discussion

Direct, invasive hemodynamic measurements such as those obtained through telemetry are considered to be the reference standard for BP measurements in canine safety pharmacology studies. In the current study, repeated simultaneous BP measurements performed by both telemetry and HDO demonstrated highly reproducible measurements for systolic, diastolic, and mean arterial BP and heart rate, although HDO-derived measurements exhibited slightly greater variability for all parameters. Of the various HDO pressure measurements, MAP exhibited the least method-dependent bias and most closely paralleled telemetric BP assessment. As such, we selected this parameter to evaluate any possible method-dependent differences in the measurement of torcetrapib-induced changes in BP. Altman–Bland agreement analysis demonstrated that with vehicle treatment, HDO measurements were characterized by a slight but significant positive bias in systolic and diastolic pressures when compared with temporally matched telemetric data. Nevertheless, torcetrapib-induced BP changes assessed by both HDO and telemetry were positively correlated and supported qualitatively similar interpretations for any possible drug-associated effects.

Blood pressure measurement by HDO is based on the Riva–Rocci principle[26] but differs from standard oscillometry by providing more rapid and sensitive measurements. Briefly, HDO measures arterial wall oscillations produced by blood flow entering an artery. When totally inflated, the cuff fully occludes the artery, halting all arterial blood flow. A custom electronic valve under computer control provides for linear cuff deflation over a range of 5 to 300 mm Hg. As the cuff pressure is decreased, blood flow reenters the artery and induces characteristic flow-dependent arterial wall oscillations, which are detected by the cuff pressure transducer over a 20- to 30-s acquisition period. A 32-bit processor allows pressure amplitude signals to be processed and displayed in real time on a computer screen. Individual pressure calculations are processed with a time lag of less than 1 µs. A proprietary Memo Diagnostic Software HDO algorithm accurately discriminates between pressure waveform changes that are characteristic of systolic, diastolic, and mean arterial pressures. Briefly, systole is defined by a unique waveform deflection that accompanies the onset of blood flow. MAP is measured directly and is defined as the period during programmed cuff deflation when individual pulse amplitudes plateau. Similarly, end-diastole is detected as a characteristic change in the pulsatile blood flow signal. Current

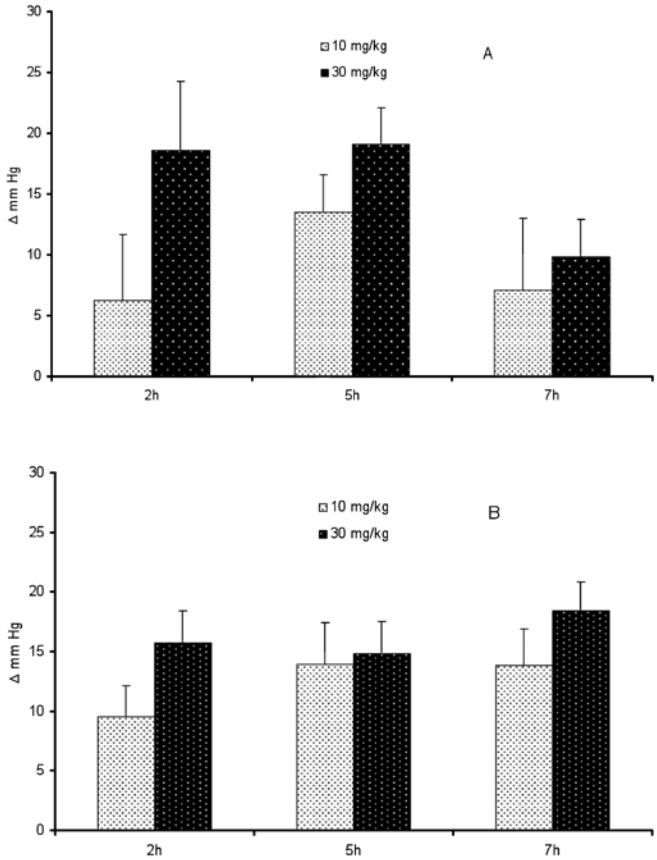

Figure 3. Changes in MAP measured by (A) HDO and (B) telemetry after torcetrapib administration. In both cases data were normalized and expressed as the individual change from the mean predose value. Although both techniques yielded generally equivalent results, the HDO values were characterized by slightly greater variability. In contrast to telemetry, HDO did not show dose-dependence by 7 h after administration.

HDO instrumentation was further optimized for use in dogs and directly measured systolic, diastolic, and mean arterial BP as well as presystolic oscillations, arrhythmias, and artifacts.

The Association for the Advancement of Medical Instrumentation scheme standards[15] require that indirect BP monitors yield measurements within 5 ± 8 mm Hg of measurements obtained by using a reference method and that 95% of indirect estimates lie within 10 mm Hg and 85% lie within 5 mm Hg of the reference measurement. These criteria were not met in the current study, but the variability between HDO and telemetric measurements may be attributed, at least in part, to the well-documented differences between central and peripheral BP measurements.[17,27] A direct methodologic comparison will require a model in which an invasive BP can be obtained in close proximity to the HDO cuff. Such methodology was beyond the scope of the current study and should be the subject of a future investigation.

Premium-quality solid-state telemetric pressure transducers may remain accurate for a brief period after implantation but subsequently are characterized by variable baseline drift.[3,23] The dogs used in this study had been implanted for 4 to 5 y, making effective baseline adjustment necessary to assure accurate pressure recordings. Moreover, because implanted telemetry pressure transducers cannot be recalibrated in situ, stable transducer gain over time must also be confirmed. According to the manufacturer, the current transducers maintain a stable and linear pressure-response over the useful life of the instrument.

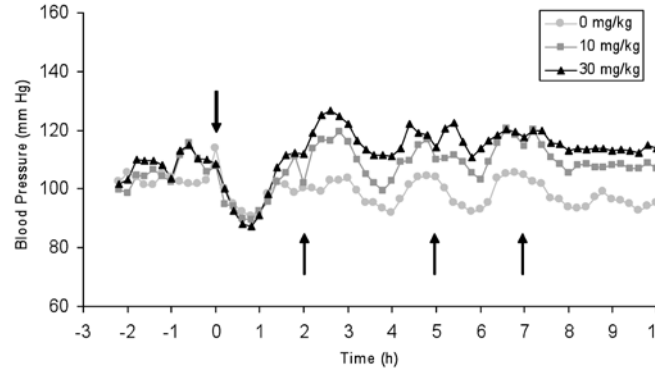

Figure 4. Effects of torcetrapib on absolute telemetric MAP. Raw telemetric MAP data exhibited a clear dose-dependent increase after torcetrapib administration (heavy black arrow). Time points at which the dogs were handled for HDO assessments (arrows; 2 and 1 h before dosing and 2, 5, and 7 h after dosing) exhibited further increases in mean arterial blood pressure. In contrast, when dogs were left undisturbed, the expected progressive decreases in blood pressure were observed.

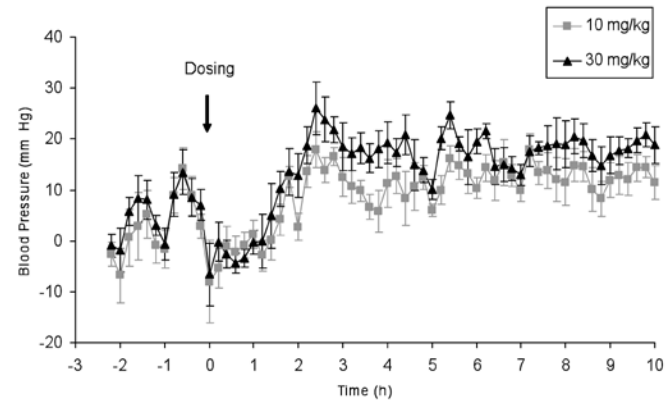

Figure 5. Normalized effects of torcetrapib on telemetric MAP. Torcetrapib administration induced dose-dependent increases in MAP, expressed as the baseline-adjusted change from vehicle. Increases in blood pressure commenced approximately 2 h after dosing (0 h). Data are provided as mean ± 1 SD.

However, to our knowledge, these performance characteristics have not been confirmed in the public domain. In the current study, baseline drift was adjusted as described over a period of 2 to 3 y. Off-treatment systolic pressures remained stable over this period (Table 1), confirming effective control of transducer drift. Moreover, pulse pressures (Table 2) also remained stable, further demonstrating that transducer gain remained stable over the observation period. All observations were made at comparable heart rates in unstressed animals that were in good health, as confirmed by a clinical veterinarian prior to data acquisition. Potentially, these observations could have been affected sporadically by unknown and uncontrolled factors, but collectively, the overall temporal stability of the values demonstrates that the current in situ calibration procedure was effective, yielding accurate and reproducible BP measurements. In addition, the historic pressure data demonstrated expected accommodation to the experimental environment, with slightly higher values noted in all subjects at 1 mo post implantation, with slightly decreased and stable values at all subsequent times.

In the current study, BP generally increased in a dose-proportional manner after torcetrapib administration, but precise characterization of possible dose-dependence was complicated by the highly variable torcetrapib exposures observed

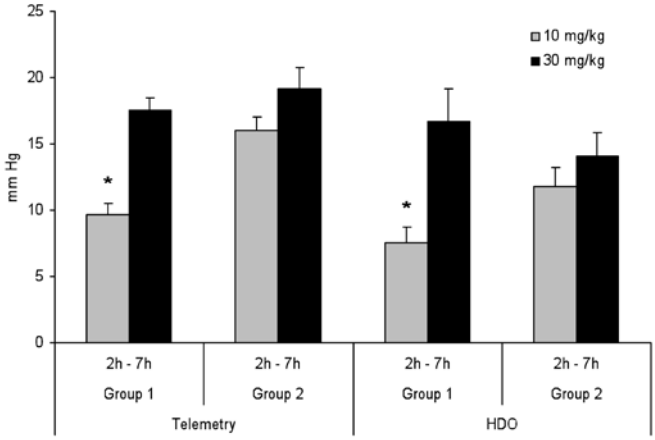

Figure 6. Effect of temperament on torcetrapib-mediated changes in BP. A dose-dependent increase in MAP was detected in group 1 (baseline MAP < 110 mm Hg) but not group 2 (baseline MAP ≥ 110 mg) dogs, demonstrating that dogs exhibiting handling-induced stress and associated elevations in baseline MAP may not be suitable for accurate assessment of dose-dependent changes in peripheral BP. The decreased HDO pressures detected at 7 h (Figure 3 A) underscore the lack of a dose-response in the 7-h data from group 2 (*, $P < 0.05$ versus value for 30-mg/kg dose).

across all subjects and doses. Moreover, because torcetrapib raises peripheral BP through indirect secondary mechanisms,[10] torcetrapib-induced BP increases do not conform to simple first-order kinetics. Collectively, the treatment-associated BP changes assessed in parallel by both HDO and telemetry were similar with respect to both magnitude and duration, as confirmed by Altman–Bland agreement analysis. Of note, the magnitude of the current telemetric MAP increase at a plasma concentration of approximately 1000 ng/mL (15.8 ± 10.4 mm Hg) was dose-proportional to the torcetrapib-mediated MAP increase recently reported (25 mm Hg at a plasma concentration of 2940 ng/mL) in a similar conscious canine model.[20] Importantly, the consistent torcetrapib exposure-pressure relationships observed in 2 separate models further supports the accuracy of the current telemetry calibration and offset procedures.

This study confirms the feasibility of obtaining reproducible and sensitive noninvasive BP measurements by HDO in dogs. HDO technology detected BP increases after torcetrapib administration that were correlated closely with parallel BP obtained by telemetry. Importantly, these data demonstrate that HDO detected drug-induced increases in peripheral BP, which were similar in magnitude to those routinely encountered in the drug-development process.

In contrast to telemetry, where data are continuously recorded while dogs are undisturbed and freely moving, HDO measurements require manual restraint of the animals to apply the cuff and to minimize motion artifacts that may be induced by tail or hindlimb movement. These characteristics render HDO measurements of BP subject to variability induced by the temperament of the individual subject during the measurement process. Behaviors such as excitement, disappointment, fatigue, and stress dynamically modulate the autonomic tone of the dogs, which is generally accompanied by catecholamine release, likely responsible for some of the fluctuations in BP and heart rate[14] observed during HDO data acquisition. In addition, torcetrapib induces the release of cortisol,[10] which has been shown to increase the sensitivity of the vasculature to catecholamines. Moreover, torcetrapib-induced BP elevation can be blocked by adrenalectomy but not by administration of a steroidogenesis inhibitor or adrenoceptor antagonists.[10] These observations suggest that factors released from adrenal glands but not aldosterone, cortisol, or catecholamines account for the BP response to torcetrapib.[10] In the current study, increased circulating catecholamine levels associated with HDO-induced stress may have potentiated the torcetrapib-induced pressure response, especially pronounced in the group 2 dogs (those whose baseline MAP was 110 mm Hg or greater). Indeed, in group 2, the torcetrapib response was maximal with the 10 mg/kg dose, effectively masking the dose-dependent nature of the response observed in group 1. Collectively, these observations suggest that dogs that exhibit pronounced handling-induced stress may not be suitable for the accurate assessment of dose-dependent changes in peripheral BP measurements obtained by using HDO methodology.

To minimize potential handling-induced influences on peripheral hemodynamics, the subjects must be trained and thoroughly acclimated to the HDO procedure. Current state-of-the-art HDO devices provide for real-time assessment of data quality. In the current study, a minimum of 6 high-fidelity HDO measurements generally was required to obtain stable BP determinations, assessed as the standard deviation of the aggregate measurements. The greatest within-subject standard deviations for repeated measurements from a single scheduled time point were 20.7, 15.7, and 16.1 mm Hg for systolic, diastolic, and mean arterial pressure, respectively. When at least 6 sequential BP measurements were performed, the mean standard deviations were reduced to 9.1 ± 4, 7.4 ± 2.8, 7.0 ± 2.7, and 7.2 ± 3.1 for systolic, diastolic, and mean arterial pressure and heart rate, respectively, demonstrating consistent but well-constrained variability. The current study used a design in which all dogs were exposed to increasing numbers of HDO procedures during torcetrapib administration. As such, any cumulative effects of experimentally induced stress likely are reflected in the sequential increases in predose pressures noted as the experiment progressed.

In typical canine toxicology studies, BP are typically not monitored due to the high variability associated with current noninvasive devices.[2,9] Due to resource constraints, dogs instrumented for telemetric data acquisition are generally not available for use in acute toxicology studies. The present study directly compared torcetrapib-induced hemodynamic changes obtained with both HDO and telemetry by using a single-dose acute experimental design. Importantly, repeat-dose toxicology studies often extend from 1 mo to as long as 12 mo and may involve different groups of animals for different dose levels of a given test item. Moreover, cardiovascular assessments typically are obtained only as infrequent 'snapshots' over the course of the experiment and then only as a single measurement within a day. Given the inherent increase in variability that is embedded within a repeat-dose toxicology study design and potential HDO measurement constraints, we feel that it is unlikely that HDO would detect BP changes with the accuracy demonstrated in the current acute setting. As such, additional long-term studies using a typical repeat-dose toxicology design will be necessary to confirm the utility of HDO in this important setting. The current results demonstrate that in an acute application, both techniques were very precise although telemetric measurements exhibited slightly greater precision. Both HDO and telemetric measurements reliably detected equivalent treatment-associated BP changes in conscious beagle dogs. Collectively, these data suggest that HDO may offer a novel and important method for the addition of noninvasive peripheral BP measurements

in canine toxicologic studies where facile and accurate BP assessments are currently not feasible.

During the conduct of this study, we noted several issues that could have affected the hemodynamic data collected. Although these factors may have influenced some of the absolute BP values obtained during the various recording sessions, we do not believe that they adversely affected the overall conclusions of the study. In the current study, HDO and telemetry measured and compared peripheral and central BP, respectively. Previous studies have demonstrated that comparisons between peripheral and central BP can be poorly correlated.[22] As such, the data from the current study provide a qualitative but not quantitative comparison between the 2 methods. Moreover, the comparison of simultaneous pressures obtained from different anatomic sites may have contributed to the positive bias with HDO pressure determinations. In addition, although torcetrapib elicits a consistent and long-lasting increase in BP, the exact mechanism of action has not been clearly elucidated. Therefore, although the ability to detect a torcetrapib-mediated BP increase is clearly of pharmacologic relevance, the variable exposures between groups and the absence of clear dose-dependence complicated interpretation of the data. The use of a pressor agent with a well characterized and direct mechanism of action will be necessary to quantitatively characterize any methodologic bias for the detection of a dose-dependent drug effect on BP. Finally, the increasing number of HDO procedures performed as the study progressed may have contributed, at least in part, to the variable baseline hemodynamics noted in this study. Possible cumulative handling effects should be addressed in a subsequent study using a Latin-Square design.

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