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Non-Invasive Spontaneous Baroreceptor Sensitivity Measurement: A Reproducible Tool to Assess Autonomic Cardiac Function in Early Drug Development

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Abstract

Spontaneous baroreflex sensitivity (sBRS) can be used to assess cardiac autonomic function. The aim of this double-blind, placebo-controlled, randomized, 3-period, crossover study was to evaluate a non-invasive methodology for monitoring sBRS as a biomarker tool in early drug development. The sBRS was determined using the ratio of the R-R interval from a 5-lead electrocardiogram (ECG) to the continuous systolic blood pressure (SBP) from a finger probe using a Finapres® NOVA device. The sBRS was measured at baseline and after two different intravenous (IV) saline and one 10 µg/kg IV atropine bolus injections in 12 healthy male subjects on 3 different study days. The sBRS changed little from baseline following saline administration (intrasubject percent coefficient of variation 14.5% [95% CI: 10.20-24.96]) and values were reproducible across the two saline administrations (Pearson's, Interclass, and Concordance Correlation Coefficient all ~0.80; Bradley-Blackwood for simultaneous test on means and variances $p > 0.93$in the morning session and $p > 0.39$ in the afternoon session). Following atropine, peak reduction in sBRS was ~56%. The pattern of the reduction was consistent with the time course of measured atropine plasma pharmacokinetics. A non-invasive methodology for monitoring sBRS is reproducible following saline and sensitive to atropine-induced changes in cardiac autonomic function in young healthy male subjects. This approach may be a valuable tool to study potential cardiac autonomic impacts of novel investigational medicinal products in early clinical development studies.

Keywords: Spontaneous Baroreflex Sensitivity; Reproducibility; Atropine; Early Drug Development

Abbreviations: sBRS: spontaneous BaroReflex Sensitivity; ECG: ElectroCardioGram; SBP: Systolic Blood Pressure; IV: IntraVenous; BP: Blood Pressure; HR: Heart Rate; CV: Coefficient of Variation; TWA: Time-Weighted Average; GM: Geometric Mean; CI: Confidence Interval; PCC: Pearson's Correlation Coefficient; ICC: Interclass Correlation Coefficient; CCC: Concordance Correlation Coefficient; PK: Pharmacokinetic; SE: Standard Error

Introduction

The arterial baroreflex is a mechanism of fundamental importance for cardiovascular homeostasis. It buffers acute changes in blood pressure through signaling between the heart and the autonomic nervous system: increased arterial pressure activates arterial baroreceptors, leading to augmented parasympathetic outflow and decreased heart

rate and vice versa. The ability of the baroreflex to efficiently buffer beat-to-beat changes in blood pressure is known as the baroreflex sensitivity (BRS). Short-term changes in blood pressure without appropriate changes in heart rate suggest impairment of the cardiac autonomic function that can be detected by measurement of the baroreceptor reflex. Therefore, quantification of the BRS can provide valuable information of cardiac autononomic regulation in normal and disease states [1,2].

Numerous techniques for the assessment of the BRS have been developed with differences in their general approaches. Older studies have used techniques in which the BRS was modulated by mechanical and chemical stimuli, including negative pressure to the neck [3], progressive lower-body negative pressure to simulate hypovolemia [4], Valsalva's manoeuvre, or administration of vasoactive drugs such as the vasodilator nitroprusside or vasoconstrictor phenylephrine [5]. More recently, the BRS was assessed under non-stimulated conditions based on the computer analysis of spontaneous blood pressure and beat-to-beat fluctuations, the so-called 'spontaneous BRS', (sBRS) [6,7]. The sBRS can be assessed using intra-arterial monitoring via catheter, an invasive method that adds additional risk to healthy volunteers in early drug studies. The sBRS can also be determined non-invasively, using the ratio of the R-R interval, derived from a single lead ECG, to the continuous systolic BP from a finger probe [8]. As this method is relatively easy and practical, it can be applied broadly and with greater convenience in clinical research settings. This non-invasive technique may be a valuable tool in early drug development to evaluate whether cardiac autonomic signaling is altered as an intended or unintended consequence of an investigational drug. However, a non-invasive approach to monitoring sBRS in a clinical trial should be supported by data that demonstrate satisfactory reproducibility (or test–retest reliability) and sensitivity to perturbations of interest under conditions expected in such a study.

The aim of this study was to evaluate a non-invasive methodology for monitoring sBRS as a potential biomarker tool in early drug development. Therefore, we assessed the reproducibility of sBRS in a small group of healthy male subjects following intravenous (IV) saline administration under the same environmental conditions on different days as well as following a bolus of IV atropine, which has been previously shown to transiently block the baroreceptor feedback loop and attenuate sensitivity in sBRS response in humans [9].

Methods

The study was approved by the local Ethics Committees and the regulatory agencies and conducted in accordance with the principles of Good Clinical Practice and the latest version of the Declaration of Helsinki. Prior to initiation of study procedures, each subject provided written informed consent.

Participants

Participants (n = 12) were non-smoking, healthy males between 18 and 45 years of age. Six of the participants were aged 18 to 30 years and six were aged 31 to 45 years. All participants had body weight less than 100 kg.

Participants were in good health based on their medical history, physical examination, 12-lead ECGs, vital signs (i.e. blood pressure (BP), heart rate (HR), oral body temperature and respiration rate) and standard clinical laboratory safety tests (hematology, blood chemistry and urinalysis). Participants with a history or clinical evidence of either syncope or Raynaud's disease were excluded. A QTc interval equal or more than 450 ms, a history of risk factors for Torsades de Pointes, hypokalemia or hypomagnesemia excluded participation in the study. In addition, participants with an injury to the middle or ring finger could not be included as one of these fingers were to be used for finger cuff measurements. All medication had to be discontinued within approximately 2 weeks or 5 half-lives of study initiation.

Study Design

The study was a single-center, randomized, placebo-controlled, double-blinded, 3-period, cross-over study in which participants were dosed either with atropine on one occasion or saline on two separate occasions. Participants were randomized into one of the three different intervention sequences over the three study visits (n=4 subjects per sequence): atropine/saline/saline, saline/atropine/saline or saline/saline/atropine. Therefore, 8 participants received saline and 4 participants received atropine in each study period. Atropine (atropine B. Braun sulphate 1 mg/ml solution for injection) was administrated as an IV bolus of 10 µg/kg atropine. An IV bolus of saline (B. Braun isotonic 0.9% saline IV solution for injection), matched for volume with atropine administration, was used as placebo control. The two administrations of saline were indicated as saline 1 (first administration) and saline 2 (second administration). Each study visit was separated by at least a 2-day washout to prevent the impact of residual pharmacodynamic effects from active drug exposure.

For each intervention visit, participants reported to the unit the evening prior to the day of study drug administration and remained in the unit until approximately 8 hours after dosing. Caffeine and alcohol were restricted for at least 12 hours

prior to each study visit and participants were instructed to avoid unaccustomed strenuous activity throughout the study. Each study visit was divided into a morning and afternoon session. Participants fasted for 8 hours prior to dosing and remained fasted throughout the morning session. Following the morning session, participants had a light lunch and were permitted to walk around the clinical research unit.

Measurements at each study visit were scheduled at the same time of the day to avoid the impact of circadian rhythms on the sBRS within and between participants. At the start of each morning and afternoon session, participants rested for 15 minutes in the semi-recumbent position (45°) to stabilize HR and BP readings, and baseline values for sBRS were determined over the final 5-minutes of rest interval. Participants were monitored in the semi-recumbent position during each session.

After receiving the IV bolus of saline or atropine in the morning, the sBRS was continuously recorded over approximately 3 hours. After the lunch break, the sBRS was continuously recorded over 20 minutes. The sBRS values were extracted at specific pre-specified time points relative to dosing during the morning (5 min, 10 min, 15 min, 20 min, 30 min, 60 min, 120 min, 3 h) and afternoon sessions (6 h 10 min, 6 h 20 min, 6 h 30 min). In the afternoon session, inhalation and exhalation were guided during the last 10 minutes, so that each participant had a consistent respiratory rate across intervention periods during this time. Each participant established his own respiratory rate, based on a comfortable respiratory rate determined prior to dosing in the morning session of the first study visit. While the guided respiratory rate could differ between participants, each participant maintained the same respiratory rate during the final 10 minutes of each afternoon assessment of the sBRS.

Measurement and Analysis of sBRS

A Finapres® NOVA 2300 device (Finapres Medical Systems, The Netherlands) was used to assess the sBRS. ECG leads were applied to the participants' chest to measure the R-R interval and a finger cuff was applied to the ring or middle finger on the non-dominant arm to measure the BP via finger plethysmomanometry. A height correction unit was applied to correct hydrostatic pressure changes due to movement in hand position relative to the heart.

The Finapres® NOVA calculates sBRS using the cross-correlation technique [10]. The algorithm calculates the cross-correlation and regression between beat-to-beat R-R interval (in ms) and SBP (in mmHg) over a sliding 10 second window, sampled at 1 Hz. The reported sBRS is the cross-correlation during the 5 minutes preceding the specified time point.

Statistical Analysis

Test-retest variability of the sBRS following saline administration was evaluated by the intrasubject coefficient of variation (CV) for the morning session and the afternoon session, separately. The primary measure of test-retest variability was based on the morning session, during which participants remained fasted and in the semi-recumbent position. For each session, a separate linear mixed effects model was used to analyze the data. The model contained a fixed effect of period and a random effect of subject. The response vector consisted of the time-weighted average (TWA) of the sBRS in natural log scale collected during the two saline periods (TWA0-3hours) for morning session and (TWA6hours 10-30minutes) for the afternoon session. The time-weighted average for any time interval is the area under the curve, divided by the time interval used. Intrasubject percent CV (calculated as 100 x sqrt(exp(s2)-1)), where s2 is the observed within-subject variance on the natural log-scale, was reported for each session. Geometric mean (GM) of TWA sBRS and the corresponding 90% confidence interval (CI) were estimated for each session and/or by study period.

In addition, the Bradley-Blackwood procedure was applied to test whether the regression coefficients in the regression of the pair-wise difference [saline 1-saline 2] versus (vs.) [(saline 1+saline 2)/2] is significantly different from 0; where saline 1 is the first dose of saline administered and saline 2 is the second dose of saline administered to the same individual. Other measures of reproducibility including the linear correlation between 2 sets of measurements (Pearson's Correlation Coefficient [PCC]), the replication reliability (Interclass Correlation Coefficient [ICC]), and the degree to which pairs of measurements coincide with a 45-degree line (Concordance Correlation Coefficient [CCC]) were calculated to assess the agreement of TWA sBRS measurements (saline 1 vs. saline 2) for each session. Scatterplots with a diagonal line were provided for TWA of the sBRS measurements (saline 1 vs. saline 2) for each session.

A linear mixed effects model appropriate for a 3-period crossover design was used to assess the effect of atropine on the sBRS. The model contained fixed-effects for intervention (atropine, saline), time (5, 10, 15, 20, 30, 60, 120 minutes, and 3 hours), period (Study Period 1, 2, 3), intervention-by-time interaction, intervention-by-period interaction, time-by-period interaction and a random effect for participant. The response vector consisted of natural log transformed percentage change from baseline in the sBRS at 5, 10, 15, 20, 30, 60, 120 minutes, and 3 hours post IV administration. An unstructured covariance matrix was used to model the

correlation between the repeated measurements on the same subject. Kenward Rodgers adjustment to the degrees of freedom was employed. The least squares mean and corresponding 90% CIs was constructed by intervention and time. Maximum reduction from baseline in the sBRS was computed for each subject across all time points and then averaged to demonstrate the effect on the sBRS in both treatment groups.

Bioanalysis and Pharmacokinetic Calculations

Plasma samples for pharmacokinetic (PK) analysis of atropine were collected up to 8 hours after dosing. Plasma atropine concentrations were determined by NMS labs (Horsham, PA, USA). Plasma samples were stored at – 20°C until the time of analysis. Atropine plasma concentrations were determined by a validated clinical diagnostic high-performance liquid chromatography–tandem mass spectrometry method with an assay range of 0.20 – 50 ng/mL for plasma.

Individual plasma concentration versus time profiles of atropine were used to generate PK parameters using non-compartmental analysis performed in Phoenix WinNonlin version 6.3 (Pharsight Corporation, St. Louis, MO, USA). The maximum concentration observed (C_{max}) and the time at which C_{max} was observed (T_{max}) were assessed by inspection of the plasma concentration data. One participant had only 1 quantifiable concentration of atropine and was excluded from all plasma pharmacokinetic calculations. Ten subjects had a quantifiable sample at 8 hours to calculate the terminal phase and were included in the $AUC_{0\text{-}8hrs}$ (area under the curve) and $t_{1/2}$ (half-life) calculations.

Tolerability and Safety

Tolerability and safety were assessed by adverse experiences monitoring and by physical examinations, vital signs, 12-lead ECGs and laboratory safety evaluation throughout the study.

Results

Twelve male participants, mean age 29.2 years (range 19 to 41 years) were enrolled and completed the study. The mean body weight was 77.5 kg with a weight range of 62.8 to 94.8 kg.

Safety

No serious adverse experiences occurred during the study. No subjects discontinued the study due to an adverse experience. All clinical adverse experiences were mild in intensity and resolved spontaneously before completion of the study. No clinically meaningful findings were observed for laboratory safety tests, vital signs or ECGs.

Nine (9) of the 12 subjects enrolled reported one or more clinical adverse experiences during the study: 5 participants (41.7%) experienced an adverse event following atropine and 6 of 12 participants (50.0%) experienced an adverse event following saline administration (in either of the two administrations) (Appendix, Table 1). A total of 4 participants (33.3 %) reported an adverse event following atropine that was considered to be intervention related. The most frequently reported (by 2 or more participants) atropine intervention related adverse events were dry mouth (n = 2) and palpitations (n=2). Following saline administration, postural dizziness was observed in one volunteer that was considered to be intervention-related.

Spontaneous Baroreceptor Sensitivity

The morning baseline sBRS (mean ± SE) was similar immediately prior. Figure 1 shows the mean percent change in sBRS from baseline (± SE) over time following both IV boluses of saline and the IV bolus of atropine. Observations of the change in sBRS following saline and atropine and described below.

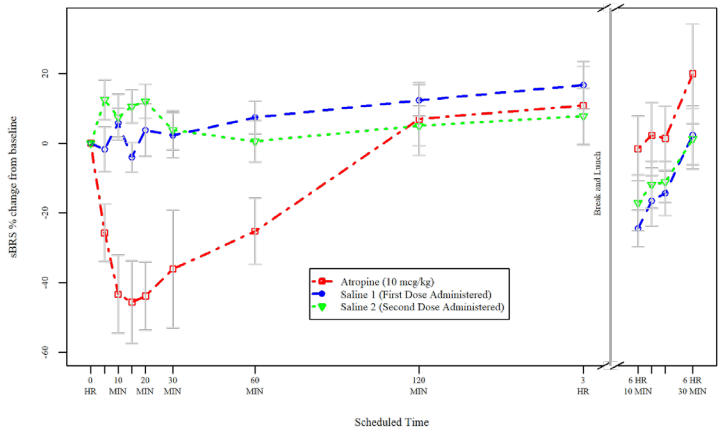

Figure 1: Mean (SE) change from baseline for spontaneous baroreceptor sensitivity (sBRS) following a single IV bolus administration of atropine (10 µg/kg) or saline to healthy male subjects (n = 12).

Following administration of IV saline, the sBRS remained close to baseline during the morning session and the pattern of change from baseline was similar in the two saline interventions. The intrasubject CV of the sBRS following a single IV administration of saline in the morning session was approximately 14.5% (95% CI: 10.20-24.96). The geometric means (90% CI) of TWA0-3h sBRS were comparable across saline interventions for the morning session, irrespective of the Study Period in which it was administered: 18.49 msec/mmHg (15.52, 22.03) in Study Period 1; 18.79 msec/mmHg (15.77, 22.38) in Study Period 2; and 18.51 msec/mmHg (15.54, 22.05) in Study Period 3 (Appendix, Table 2). Further supporting similarity between the two saline intervention responses, the correlation coefficients (ICC, PCC, and CCC) were high (Table 1) and the p-value was >0.90 for the Bradley-Blackwood test-retest variability procedure (Appendix, Table 3).

Session	ICC	PCC	CCC
Morning	0.789	0.817	0.815
Afternoon	0.8	0.803	0.773
CCC=Concordance Correlation Coefficient; ICC=Intraclass Correlation Coefficient; PCC=Pearson's Correlation Coefficient			

Table 1: Correlation measures of sBRS following two different saline administrations.

In the afternoon session, the sBRS followed a similar pattern in saline 1 and saline 2 interventions. The initial afternoon assessment (6 hour 10 min) in both saline administrations appeared to be lower than the morning baseline value, and sBRS increased systematically over the afternoon session in both saline administrations. TWA of natural log sBRS measurements were in agreement in both morning and afternoon assessments across saline 1 and saline 2 administrations as supported by the scatter plot displayed in Figure 2.

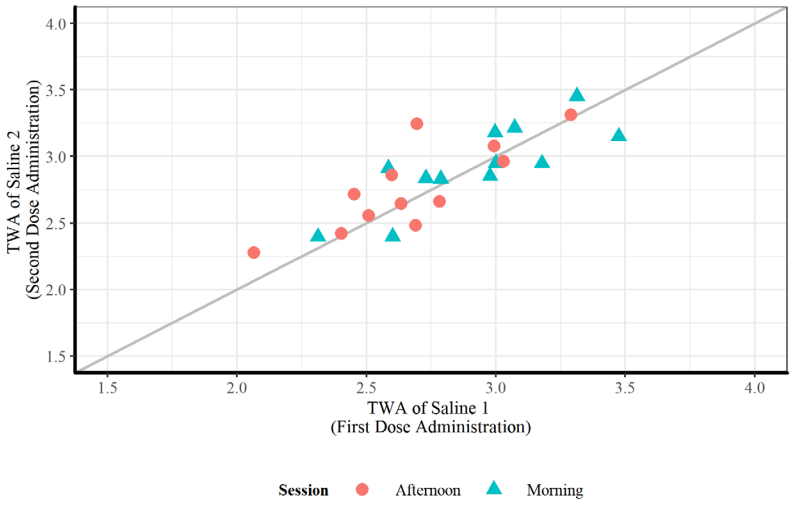

Figure 2: Time weighted average (TWA) of natural log spontaneous baroreceptor sensitivity (sBRS) following saline administration to healthy male subjects (n=12) (saline 1 versus saline 2).

Following IV atropine administration, the sBRS fell rapidly and returned to baseline before the end of the morning session. The mean peak reduction from baseline sBRS following atropine administration at any time following dosing was 56.56%, which is much larger than the mean peak reduction of ~17% observed following either saline administrations at any time following dosing (Table 2). When examined in fixed intervals from the time of dosing (Table 3), the maximum percentage reduction from baseline sBRS was observed between 10 to 30 minutes after administration of atropine. As observed by the LS means difference, the reduction was highest in the same time period (10 to 20 minutes) relative to saline too. The pattern of this reduction in sBRS is consistent with the anticipated rapid onset and

short elimination half-life of atropine.

Treatment	Maximum Reduction from Baseline % (± SE)
Atropine (10 µg/kg)	-56.56% (-63.47, -49.66)
Saline 1	-16.83% (-20.65, -13.02)
Saline 2	-16.56% (-18.97, -14.16)
Baseline (Time point 0 hour); SE=Standard Error	
Saline 1=the first saline dose administered; Saline 2=the second saline dose administered	

Table 2: Maximum reduction from baseline in sBRS following atropine or saline administration (morning session only).

Time	LS Means (90% CI) of Atropine	LS Means (90% CI) of Saline	Difference in LS Means Relative to Saline (90% CI)
0 hour	-0.00 (-11.68, 11.68)	-0.00 (-8.26, 8.26)	0.00 (-14.30, 14.30)
5 minutes	-25.74 (-37.42, -14.07)	5.35 (-2.90, 13.61)	-31.10 (-45.40, -16.80)
10 minutes	-43.35 (-55.02, -31.67)	6.65 (-1.61, 14.91)	-50.00 (-64.30, -35.69)
15 minutes	-45.59 (-57.27, -33.91)	3.25 (-5.01, 11.50)	-48.84 (-63.14, -34.54)
20 minutes	-43.87 (-55.54, -32.19)	7.87 (-0.39, 16.13)	-51.74 (-66.04, -37.44)
30 minutes	-36.09 (-47.77, -24.42)	2.96 (-5.30, 11.22)	-39.05 (-53.36, -24.75)
60 minutes	-25.25 (-36.92, -13.57)	3.94 (-4.31, 12.20)	-29.19 (-43.49, -14.89)
120 minutes	6.89 (-4.78, 18.57)	8.61 (0.36, 16.87)	-1.72 (-16.02, 12.58)
3 hours	10.80 (-0.87, 22.48)	12.20 (3.95, 20.46)	-1.40 (-15.70, 12.90)
CI=Confidence Interval; LS Mean=Least Squares Means; sBRS expressed in msec/mmHg.			

Table 3: Percentage change from baseline sBRS (LS Means) following IV atropine or saline by timepoint (morning session only).

In the afternoon session following atropine administration, the initial assessment (6 hour 10 min) appeared to be lower than the morning baseline value and final value in the morning session, and sBRS increased over the afternoon session, as was also observed in both saline administrations. The intersubject CV of the sBRS following atropine administration in the morning and afternoon are approximately 15% and 12%, respectively.

The summary of atropine PK parameters following IV administration is provided in Table 4. The results of sBRS change from baseline are consistent with the time course of atropine pharmacokinetics (see Figure 3). There was a statistically significant relationship between atropine concentration and percent change in sBRS from baseline at the sampled time points (Supplemental Figure 1).

Analyte	Dose (µg/kg)	N[a]	AUC0-8 (hr*ng/ml)[b]	Cmax (ng/ml)	Tmax (mins)[c]	t½ (h)[b]
Atropine	10	11	8.4 (21.5)	3.92 (29.1)	5 (5 - 30)	2.67 (13.6)
AUC0-8: area under the curve from time 0 to 8 hours						
[a] One subject had only one quantifiable concentration and was excluded from all summary statistics.						
[b] Only 10 subjects had quantifiable sample at 8 hours to calculate terminal phase and were included in AUC0-8 and $t_{1/2}$ summary statistics.						
[c] Median (minimum-maximum)						
Non-compartmental analysis completed using nominal times.						

Table 4: Summary of atropine plasma pharmacokinetic parameters [geometric mean (geometric Coefficient Variation %)] following a single intravenous bolus dose of atropine (10 µg/kg) to Healthy Male Subjects.

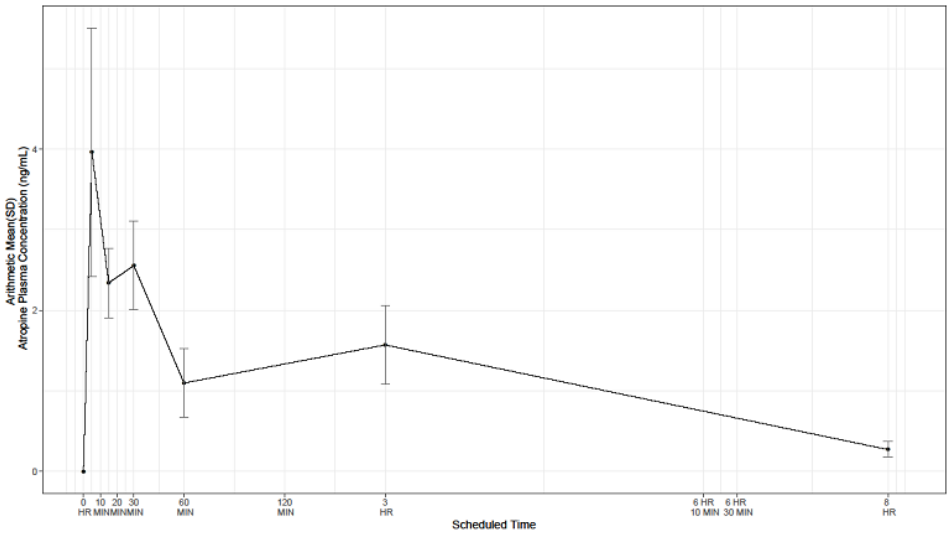

Figure 3: Arithmetic mean (SD) plasma concentration versus time profile of atropine (10 µg/kg) following IV bolus administration to healthy male subjects (n=11).

Discussion

Primary cardiovascular safety parameters such as blood pressure and electrocardiogram may not be sufficiently sensitive to identify drug effects on the baroreflex function. In conscious, telemetered rhesus monkeys, compound-dependent effects on heart rate variability and sBRS have been observed at doses that had little or no effect on the spontaneously recorded individual values for BP and HR [11]. Therefore, additional assessment of the sBRS in early clinical drug development may be important to detect possible drug-related cardiovascular effects that are otherwise 'silent' to standard hemodynamic vital sign monitoring but may have implications for safety or tolerability, depending on the mechanism. Consequently, a well-validated and practical methodology for quantifying the sBRS may provide an early means to assess safety or tolerability risks in a well-monitored setting.

Our study demonstrated that the non-invasive Finapres® NOVA system can reliably measure sBRS in healthy male participants under conditions similar to those in an early clinical drug development trial. Changes in sBRS were small and reproducible following saline administration on different days across both morning and afternoon sessions. The intra-subject CV was found to be lower than 25%, a result consistent with prior reports [9,13,14]. Therefore, the method evaluated in this study would be expected to have sufficient sensitivity to reliably report sBRS changes >25% from baseline.

The methodology was sensitive to transient reductions in sBRS induced by atropine. The pattern, magnitude, and duration of the sBRS reduction following 10 µg/kg IV atropine in our study was consistent with expected pharmacological properties of atropine, reflected measured atropine concentrations in the blood concurrent with sBRS measurement, and were similar to those in an earlier report using an invasive method of sBRS detection [9]. We and others [9] have now demonstrated reductions of sBRS approaching or exceeding 50% of baseline following atropine. While the clinical significance of this magnitide of sBRS change is unclear, the 50% reduction provides a benchmark against which the impact of other interventions can be compared.

Notably, good reproducibility of the method following saline administration and the sensitivity to changes during atropine administration was obtained in 12 healthy male participants. This sample size and population are typical of early clinical development trials, and our results therefore demonstrate this method can be used reliably in comparable sized studies.

Additional observations from the study deserve consideration and may suggest approaches to further optimize the assessment of sBRS in early clinical development studies. Although both morning and afternoon baseline assessments were conducted during the final 5 minutes of a 15-minute rest, the initial afternoon assessment (6 hour 10 minutes) tended to be lower than the morning baseline assessment. Allowing participants to have lunch and move around between morning and afternoon sessions may have contributed to this difference. Other potential explanations for this pattern could include random variation or diurnal cycles in sBRS. Why any of these factors would have a more pronounced impact on the 6 hour 10 minutes assessment during the two saline intervention periods than during the

atropine intervention period is unclear, particularly since the effects of atropine on sBRS appeared to have resolved prior to the end of the morning session. Based on these observations, we recommend maintaining semi-recumbent positioning and fasting state throughout the most critical assessments of the sBRS.

In addition, sBRS was observed to increase during the final 10 minutes of the afternoon sessions during both placebo intervention visits and, to a lesser extent, during the atropine intervention visit. This may have reflected continued stabilization of sBRS following the lunch break, as discussed above. However, it may also have been impacted by the implementation of guided respiration during the final 10 minutes of the afternoon session, which occurred in all intervention periods. It is known that the sBRS is influenced by the breathing frequency because respiration affects the physiological oscillations which modulate BP and HR [9]. Slow breathing has been shown to increase sBRS in patients with essential hypertension [12]. However, healthy subjects have a slow, regular respiration rate at rest that does not vary greatly over time [13]. In this study, the sBRS measurements were reasonably reproducible across the saline assessments during the intervals of both free respiration (morning session and early afternoon session) and guided respiration. Therefore, we propose that the change in sBRS can be assessed in healthy volunteers without the necessity for guided respiration.

We note some limitations to the current study. We studied the sBRS in healthy males aged 18-45 years as this group is often enrolled in early clinical drug development studies. However, testing the methodology and understanding its performance in subjects from other demographic groups could be of interest. Studies have demonstrated that the sBRS is similar in males and females [14] but decreases in sBRS have been observed with age [15,16]. Furthermore, the study was conducted in normotensive subjects. Increased blood pressures in hypertensive patients have been shown to reduce the BRS [17]. Therefore, it may be necessary to understand the methodology capabilities in patients with hypertension and other cardiovascular co-morbidities before applying this approach in early clinical development trials in patients.

Conclusion

In conclusion, our study demonstrates that a non-invasive technique for measuring sBRS is reproducible and sensitive to atropine-induced changes in cardiac autonomic function when tested in a small sample of healthy young male subjects. Therefore, this approach may be a valuable tool to investigate the potential cardiac impact of novel agents in early clinical development studies.

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Conflict of Interest

This study was funded by Merck Sharp & Dohme Corp., Whitehouse Station, NJ, USA. The following authors are employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA: AD, HN, AM, AS, SAS, LA, RD, IDL, GAW

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