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Monitoring haemodynamic changes in rodent models to better inform safety pharmacology: Novel insights from *in vivo* studies and waveform analysis

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Abstract

Animal models are essential for assessing cardiovascular responses to novel therapeutics. Cardiovascular safety liabilities represent a leading cause of drug attrition and better preclinical measurements are essential to predict drug-related toxicities. Presently, radiotelemetric approaches recording blood pressure are routinely used in preclinical *in vivo* haemodynamic assessments, providing valuable information on therapy-associated cardiovascular effects. Nonetheless, this technique is chiefly limited to the monitoring of blood pressure and heart rate alone. Alongside these measurements, Doppler flowmetry can provide additional information on the vasculature by simultaneously measuring changes in blood flow in multiple different regional vascular beds. However, due to the time-consuming and expensive nature of this approach, it is not widely used in the industry. Currently, analysis of waveform data obtained from telemetry and Doppler flowmetry typically examines averages or peak values of waveforms. Subtle changes in the morphology and variability of physiological waveforms have previously been shown to be early markers of toxicity and pathology. Therefore, a detailed analysis of pressure and flowmetry waveforms could enhance the understanding of toxicological mechanisms and the ability to translate these preclinical observations to clinical outcomes. In this review, we give an overview of the different approaches to monitor the effects of drugs on cardiovascular parameters (particularly regional blood flow, heart rate and blood pressure) and suggest that further development of waveform analysis could enhance our understanding of safety pharmacology, providing valuable information without increasing the number of *in vivo* studies needed.

Keywords

Radiotelemetry, Doppler flowmetry, safety pharmacology, waveform analysis

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Introduction

Over the last few decades, cardiovascular safety pharmacology concerns, comprising of direct and indirect toxic effects on the heart and vasculature, have been a major contributing factor to drug-attrition in all stages of drug development.[1] Often, this drug-associated cardiovascular toxicity is used to describe toxicities observed in all components of the cardiovascular system such as the heart, vasculature and blood components.[1,2] These toxicities demonstrate detrimental effects in both function and structure of one or more of the components of the cardiovascular system.[1,2] To minimise the risk of therapy-associated cardiovascular complications, better preclinical models are needed to improve the translatability of preclinical findings into clinical practice.[2]

Currently, drug safety assessments are conducted in accordance with the guidelines of the International

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Conference on Harmonization (ICH) S7A and S7B.[3,4] Notably, *in vitro* detection of human Ether-a-go-go Related Gene (hERG) potassium channel block has proven relatively effective in identifying major drug-induced cardiotoxicity.[2,4] Additionally, *in vivo* cardiovascular investigations for changes in heart rate (HR) and blood pressure (BP), are typically performed in rodents and in larger non-rodent mammals using radiotelemetric approaches.[3–5] Follow-up studies include cardiac output and ventricular contractility measurements.[3,6] Despite this, the high attrition rate of pharmaceutical candidates due to cardiovascular safety liabilities remains, highlighting the limitations associated with current preclinical studies.[1] Some of these limitations, due to the inability to determine the mechanism behind *in vivo* adverse reactions or to predict longer-term detrimental effects in patients,[2,7] can lead to attrition of novel therapeutics late in clinical development, contributing to substantial drug development delays and increasing the overall cost of bringing new drugs to market.[2,7]

Although the limited clinical translatability of animal models inherently contributes to drug attrition, the utility of robust preclinical *in vivo* approaches has also been reported.[2,8] Physiologically relevant cardiovascular monitoring beyond BP enables the detection of integrated regional haemodynamic responses in conscious rodents.[9,10] These data provide novel information about the mechanisms responsible for specific cardiovascular responses and may help explain and prevent the unexpected cardiovascular events seen in patients.[9,10] As an example, an emerging challenge is represented by cardiovascular toxicity from novel molecular-targeted cancer therapies, such as receptor tyrosine kinase inhibitors (RTKIs),[11,12] whose implications in cardiovascular impairment are still far from clearly understood.[13] Comprehensive haemodynamic measurements, beyond BP monitoring alone, are starting to expand our understanding of the mechanisms underlying this cardiovascular toxicity[14] and could more broadly serve as an approach to improve drug safety.

This review discusses the importance of regional haemodynamic measurements from preclinical assessments in small laboratory rodents and how waveform analysis of cardiovascular recordings could help predict and prevent cardiovascular toxicities in humans. The importance of radiotelemetry will be summarised, and the Doppler flowmetry approach discussed. The latter technique allows for the continuous recording of Doppler shift (an index of blood flow), concomitantly with changes in mean arterial pressure (MAP) and HR, in conscious, freely-moving rats.[9,10,14] Since regional haemodynamic changes may precede or may not always be apparent from MAP and HR measurements alone, this model provides mechanistic insights into integrated cardiovascular responses to novel interventions, which may be regionally selective, temporally distinct, and sometimes opposing in different vascular beds.[9,10] Findings from both telemetric and Doppler flowmetry approaches will then be discussed in the context of waveform analysis, as a potential future strategy for predicting cardiovascular liabilities with better precision.

Radiotelemetry

Radiotelemetry in conscious, freely-moving animals is a widely accepted method to evaluate the haemodynamic effects of a drug candidate during preclinical studies.[6,15] Haemodynamic reflexes and indirect cardiovascular responses, that might be absent in anesthetised animals, are intact. Therefore measurements from conscious animals are likely to be representative for cardiovascular effects occurring in clinical settings.[6,15] Small, implantable radiotelemetric devices for research animals are commercially available, allowing for the wireless recording of physiological variables such as BP and HR, but also electrocardiogram, electroencephalogram, body temperature and physical activity.[15,16] Radiotelemetry in rats is routinely used during the preclinical stages of drug development[3,17] as a valuable tool for predicting the safety profile of therapeutics in humans.[15,16]

To monitor cardiovascular variables, a pressure sensor is implanted in the carotid artery or abdominal aorta.[18] A radiotelemetry receiver is placed directly beneath each cage and receives the radio signals from the implanted transducer (Figure 1). The consolidation data exchange matrix then transfers the digitised signal to the data acquisition system.[15,16]

In comparison to traditional methods of BP monitoring, two main advantages of radiotelemetry include a better data quality and advancements in animal welfare.[15,16] For example, tail-cuff manometry, another method for measuring BP, requires restraint of the animal and interaction with a researcher, leading to animal distress and consequent alterations of BP, HR and body temperature, therefore producing artefacts in the recorded data.[15] In contrast, radiotelemetry data are collected in freely moving and conscious animals, without interaction with the researcher, minimising stress.[15] Data collection from undisturbed animals in their home cages is preferable, as these conditions are representative of the normal state of the animal, and the data have, therefore, a higher predictive value.[15–17] Telemetry recordings can be conducted from socially housed rats, further improving animal welfare (Figure 1).[17]

Moreover, this approach reduces the number of animals used as multiple variables can be recorded from one animal, decreasing the need for separate studies.[15,16] Additionally, animals implanted with telemetric devices may be reused in separate, consecutive studies due to the long lifetime of the implant's battery.[15]

In addition to short-term data, radiotelemetry is an ideal tool for assessing long-term cardiovascular consequences and can accurately recapitulate clinical conditions in rodents.[15,16,18] Mills et al. (2000) described the possibility to record in unrestrained mice for up to 150 days.[19] Cooper et al. (2019), evaluated RTKI-induced BP

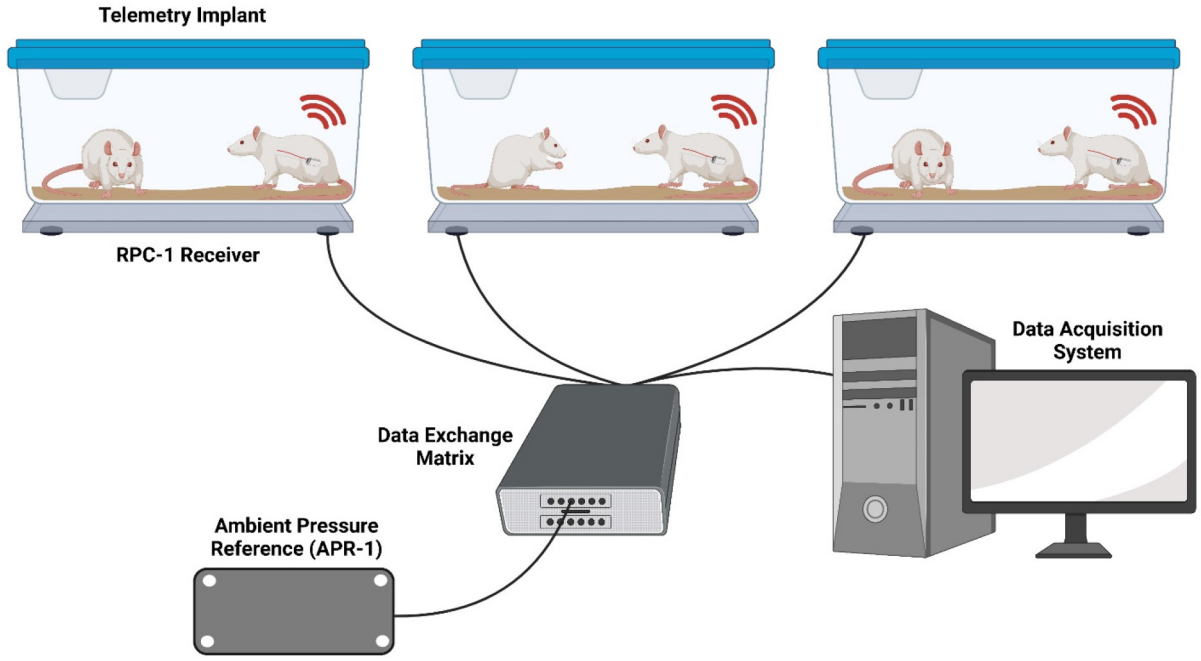


Figure 1. General set-up of a radiotelemetry study. Animals are generally co-housed with either an un-instrumented 'buddy' or, in more recent multi-channel systems, with another instrumented rodent. Created with Biorender.com.

changes in telemetered rats during 21 days of treatment, followed by 10 days without treatment,[18] and were able to recapitulate BP elevations in this model.[18] Moreover, sustained hypertension was observed after cessation of treatment, corroborating the long-term cardiovascular implications following VEGF-inhibition observed in clinical settings.[18]

Despite the many advantages over other approaches, radiotelemetry studies require specialised training for surgical procedures and experiments.[15] Additionally, telemetry equipment purchase and maintenance can be expensive.[15,16] However, the expenditure may be partially offset through the reduction in the number of animals used, particularly as new systems allow for multiple instrumented animals in a single cage.[15,20]

Although offering valuable information on drug-induced cardiovascular effects, radiotelemetry is mostly restricted to monitoring BP and HR. Pharmacological agents can, however, affect peripheral haemodynamic variables (e.g. regional vasoconstrictions or vasodilatations) without evoking a significant change in overall BP.[9,10] These changes may go undetected when evaluating cardiovascular safety using radiotelemetry. As a result, a method able to measure the regional effects of a drug on distinct vascular beds is crucial to obtain comprehensive information on the treatment-induced response.

Pulsed Doppler flowmetry

The pulsed Doppler flowmetry was first described nearly 50 years ago by Hartley and Cole, who developed an *in vivo* approach for measuring regional blood flow in dogs.[21] Whereas traditional electromagnetic probes had proven difficult to calibrate and were both bulky and heavy, the development of a pulsed Doppler system, requiring a single piezoelectric crystal capable of both emitting and receiving pulsed ultrasonic energy, was thus a significant advancement.[21] The approach was further developed by Haywood et al. for use in rodents[22] and refined by the haemodynamics team of Gardiner and Bennett at the University of Nottingham.[23] Their surgical approach allows for implantation of up to three Doppler flow probes for simultaneous measurement of changes in vascular conductance in several regional beds, including the renal, superior mesenteric, and hindquarters vascular beds (Figure 2).[9,10] In addition, the implantation of intra-arterial catheters to monitor MAP and HR allows for the continuous measurement of haemodynamic variables (Figure 2).[9,14]

The implantation of the probes and catheters involves a two-stage surgery. During the first stage, a piezoelectric crystal, emitting a short burst of pulsed ultrasonic energy and detecting the signal reflected by moving erythrocytes, is positioned on the appropriate vessels as described previously (Figure 3).[9,10,24] The reflected energy is at a slightly shifted frequency relative to the transmitted signal; this is referred to as the 'Doppler shift' and is proportional to the velocity of the erythrocytes, therefore reflecting the blood flow velocity in the downstream vascular bed.[9,21,22]

Following probe implantation, a 10-day recovery period is required prior to performing the second surgery, which

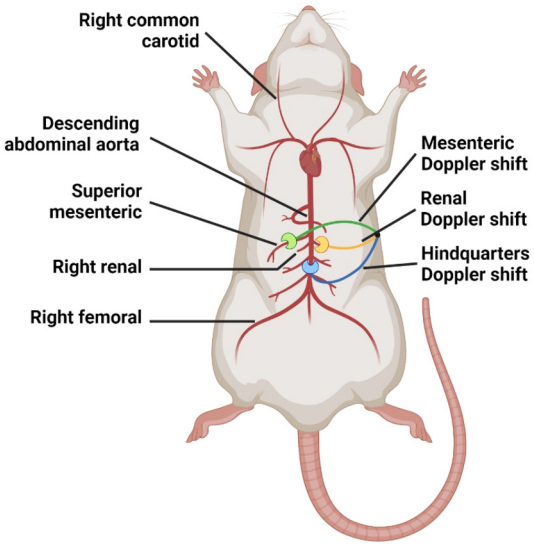


Figure 2. Surgical implantation of Doppler flow probes. Rat in supine position showing a schematic arterial network, with the positioning of Doppler flow probes around the superior mesenteric and left renal arteries and descending abdominal aorta (highlighted in green, yellow and blue, respectively). Created with BioRender.com.

consists of the implantation of catheters into the right jugular vein (for drug administration) and a catheter into the distal abdominal aorta to monitor MAP and HR.[9,10,24]

Contrary to wireless recording in telemetry studies, animals are tethered during the recording of the Doppler data. A harness with a flexible spring and counterbalance arm is placed over the rat's head, and the catheters are passed through the spring and connected to a Doppler flowmeter and BP transducer (Figure 4).[9,10,24] Animals are given analgesia and allowed to recover for 10 days after the probe implantation surgery. The catheter to measure blood pressure changes is then placed via the tail artery into the distal abdominal aorta. Experiments begin approximately 24 h after the second surgery. Animals are singly-housed on experimental days.[10,14,24] Wauschkuhn et al. (2005) have also used Doppler flow measurements combined with radiotelemetry to monitor cerebral blood flow.[25] Previous telemetry measurements[18,25] have shown circadian rhythms in HR and BP. For this reason, Doppler experiments are normally conducted at the same time every day.

Haemodynamic measurements of HR and BP, together with renal, mesenteric, and hindquarters Doppler shifts, are recorded using bespoke software (IdeeQ; Maastricht Instruments, version 2.5, Maastricht, The Netherlands) (Figure 5A). The BP waveform analysis and the Doppler shift waveform analysis are executed using the same algorithm in IdeeQ (Figure 5B). First, initialisation is carried out using the first two seconds of the waveform signal. Using the cardiac cycles in this period, the maximum slope (S_{max}) of the signal is determined by means of differentiation (W_{diff}). After this, the signal is checked for beats (i.e. cardiac cycles) until the end of the curve. For one cardiac cycle, the start of the beat (t_s) is detected when the slope of the curve exceeds a set percentage of the average S_{max} of the last 8 beats, and the curve exceeds the average of the curve at two consecutive moments in time (t_1 and t_2). (Figure 5B) Similarly, the end of the cycle (t_e) is detected when the slope of the curve exceeds a set percentage of the average negative maximum slope, and the curve is lower than the average value of the curve. (Figure 5B) In this way, all beats of the signal are detected. The end-diastolic pressure (P_{ed}) or flow is defined as the minimum value of the curve prior to the start of the beat, the systolic pressure (P_s) or flow is defined as the maximum value of the curve before the slope of the curve turns negative. MAP, Doppler shift and vascular conductance are calculated as shown in equation 1 – 3 (Figure 5C).

Pulsed Doppler flowmetry allows for the measurement of drug-induced regional haemodynamic effects in conscious, freely-moving rats, with the advantage of observing such responses in the presence of intact baroreceptor afferents, along with other neuronal/neurohormonal influences and autoregulatory responses.[10,23] As demonstrated many times, different vascular beds can respond differentially, and sometimes in opposing ways.[9,10,14] In such a situation, an intervention may cause a negligible change in MAP and yet cause substantial vasoconstrictor effects in some vascular beds, together with vasodilator effects in other vascular beds. This information is critical for understanding integrated cardiovascular regulation *in vivo*[9,10,14,24] and currently cannot be modelled *in vitro*.

Carter et al. (2017), evaluating the RTKI-induced haemodynamic consequences in rats implanted with pulsed Doppler flow probes,[14] reported an hypertensive effect associated with these drugs, that was consistent with clinical findings,[13] and was associated with vasoconstriction in the hindquarters for all RTKIs studied.[14] This study, monitoring the effects on distinct vascular beds and delivering information about the haemodynamic profile of RTKIs alongside BP and HR, helped to understand the mechanisms underlying RTKI-induced cardiovascular toxicities.[14]

Further waveform analysis of Doppler flowmetry data could provide earlier detection of haemodynamic changes and mechanistic insight into the development of drug-mediated cardiovascular events.

Waveform analysis

Current telemetry and flowmetry approaches are able to detect clear changes in cardiovascular variables.[26] However, more subtle cardiovascular changes, which may be predictive of longer term adverse events, often go

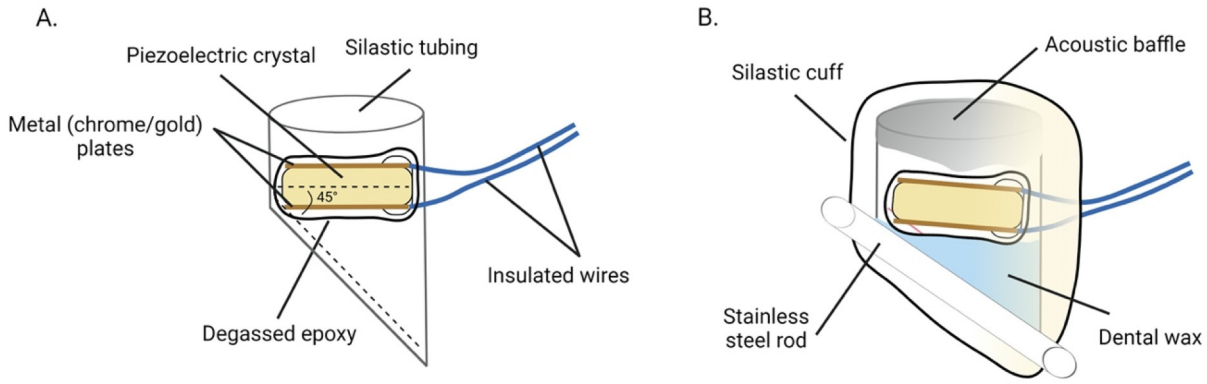


Figure 3. Cuff development of the miniaturised pulsed Doppler flow probe. Schematic representation of the miniaturised Pulsed Doppler flow probe made in-house based around the design given in (Haywood et al., 1981).[37] (A) A 1 mm diameter 20 MHz piezoelectric crystal with insulated copper wires is housed inside silastic tubing, designed to sit at a 45° angle to the blood vessel lumen. (B) The back and sides around the crystal are covered with an acoustic baffle to prevent interference from surrounding blood vessels. A soft silastic cuff is created with the aid of a section of stainless steel tubing to encase the probe and allow the creation of a lumen for the vessel to sit in. Adapted from Haywood et al. 1981.[37] Figure created with BioRender.com.

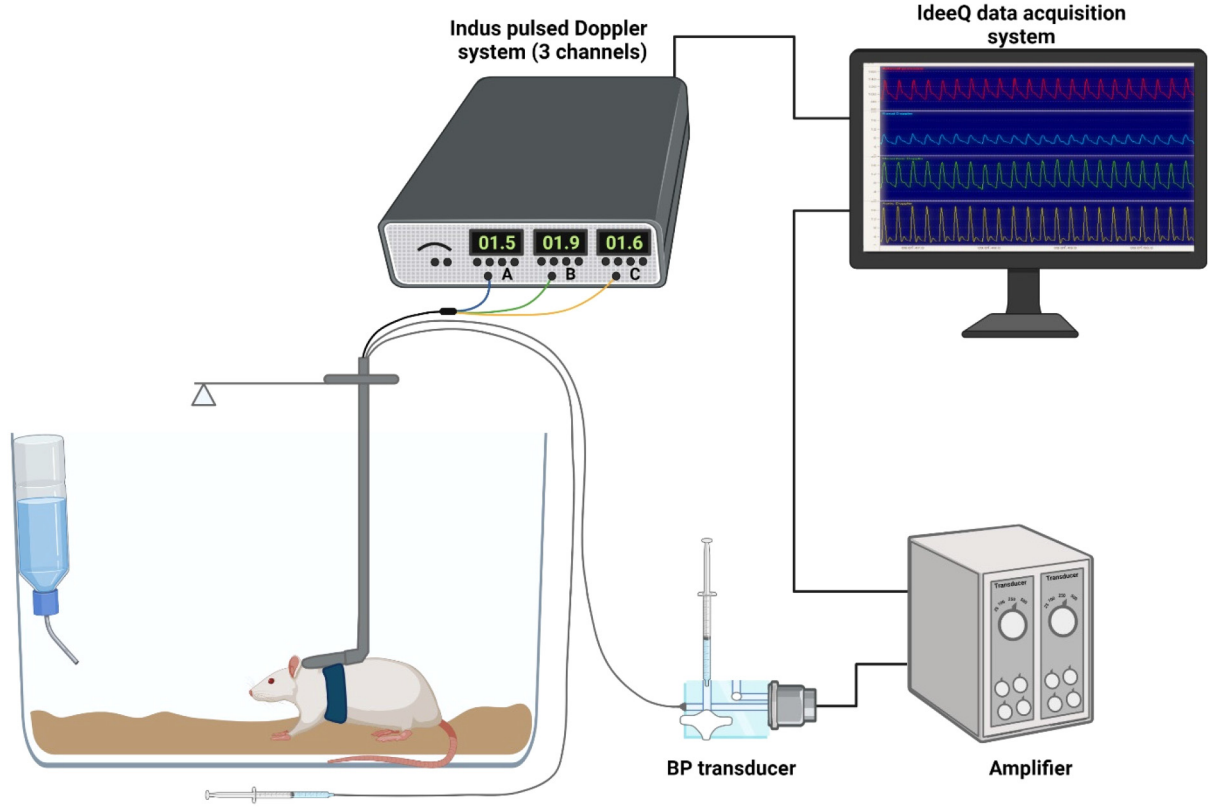
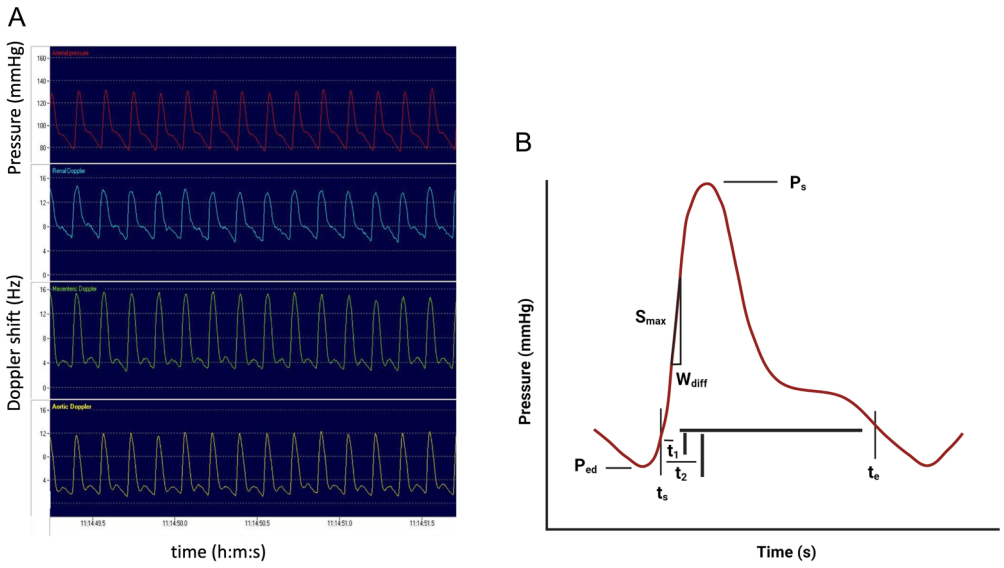


Figure 4. Doppler flowmetry experimental set-up. Single-housed rats, instrumented with Doppler flow probes around the superior mesenteric and left renal arteries, and descending abdominal aorta (highlighted in green, yellow and blue, respectively). Additional intravascular catheters, including intravenous catheters for drug administration and an arterial catheter which is connected to the blood pressure (BP) transducer for measurement of heart rate and mean arterial pressure are also present. Amplified signals are digitalised and recorded using an IdeeQ data acquisition system. Figure created with BioRender.com.

undetected.[6,26] The question remains concerning whether the currently available assays are sensitive enough to detect small changes in BP which, if persistent, may lead to dose-limiting toxicities and drug attrition.[2,26] Although BP in rodents is generally seen as a biomarker that is qualitatively translatable to clinical studies, quantitative



C

$$MAP = \frac{2 * DBP + SBP}{3} \quad (1)$$

Where ***MAP*** = Mean Arterial Pressure, ***DBP*** = Diastolic Blood Pressure and ***SBP*** = Systolic Blood Pressure

$$\Delta f = 2fo\frac{v}{c}\cos\theta \quad (2)$$

Where Δf = Doppler shift, fo = transmitted frequency in Hz, v = velocity of fluid, C = velocity of sound in the fluid, θ = angle between the flow axis and acoustic axis (the angle of the crystal in relation to the blood vessel), the difference between the frequency emitted and the frequency returned can be calculated.

$$Vascular\ Conductance\ (flow) = \frac{Mean\ \Delta f}{Mean\ MAP} \quad (3)$$

Figure 5. Example and derivation of Doppler waveforms. (A) Example recording of BP in distal abdominal aorta (red trace) and Doppler shift in renal (blue), mesenteric (green) and aortic (yellow) vascular beds in IdeeQ. (B) The algorithm shows how peak systolic (P_s = SBP) and peak end-diastolic BP (P_{ed} = DBP) are measured for one cycle of the signal. (C) Equations used to calculate mean arterial pressure (1), Doppler shift (2) and vascular conductance (3). Figure 5B created with BioRender.com.

prediction of long-term cardiovascular risk in patients is still a challenge.[26] Cardiovascular complications such as heart failure and myocardial ischaemia are often only detected once drugs have been approved for clinical use, indicating the failure of current approaches to detect these toxicities during preclinical and clinical development.[26] More sensitive preclinical systems to detect subtle changes in cardiovascular parameters and to translate BP changes into long-term risk in humans would improve our capability in safety testing.[26]

In the models described above, BP and derived blood flow measurements are captured as periodic waveforms. In the current analysis of these data, peak values (P_s and P_{ed}) or single point averages (e.g. S_{max}) are typically evaluated (Figure 5B). Although these values allow for the calculation of important haemodynamic variables, this approach overlooks potential information 'hidden' in perturbations to the waveform morphology and variability. In-depth characterisation of recorded waveforms may provide more extensive information on the condition of the heart and vasculature when exposed to pharmacological agents.[27]

Past radiotelemetric studies have made some progress in moving beyond examination of mean sample data of BP and other cardiovascular variables.[28,29] For example, Anderson et al. (1999) described the use of a non-parametric regression analytical approach to examine cyclic components of BP readouts, which identified trends in variation of pressure waveforms.[28] This approach was able to distinguish the hypertensive effects of different drugs, including losartan or hydralazine + hydrochlorothiazide, by detecting differences in BP variability amplitude.[28] In a separate study, Cerutti et al. (2001) tested the validity of pulse-contour methods and compared novel multivariate statistical analyses involving a large number of parameters extracted from the pressure waveform, with the aim of

accurately estimating stroke volume.[29] Moreover, similar methodologies applied on blood flow waveforms are being explored to identify organ specific drug effects (e.g. waveform separation in forward and reflected waves).[30] These studies highlighted the wealth of information available in cardiovascular waveforms and demonstrated that these models can be applied to obtain more precise conclusions.

Other mathematical models intending to utilise all provided data and understand the complexity of physiological waveforms have been designed.[31] These analyses are particularly important in clinical settings, where applications of waveform analysis have received increased attention over the last two decades.[32] A well-established type of waveform analysis is HR variability (HRV). HRV extracts the variation in HR over time.[33,34] This popular, non-invasive analysis, reflecting the ability of the heart and vasculature to adjust to changing circumstances,[33] has been proven a valuable tool in the diagnosis and prognosis in several pathologies (e.g. diabetic neuropathy), where a low beat-to-beat variability serves as an early marker for disease progression; a reduction in HRV has been associated with an increased risk of cardiac events.[33,34]

In addition to the analysis of beat-to-beat variability, waveform morphology can be used to provide clinically useful information. For example, pulse pressure (PP, i.e. the difference between systolic and diastolic BP) and pulse pressure amplification (PPA, i.e. the ratio of brachial PP to central PP) reflect changes in waveform morphology (Figure 6).[32,35] As detailed in Figure 6, arterial and peripheral waveforms differ in both shape and amplitude. Arterial PP is an important determinant of the pulsatile load on the left ventricle (LV).[36] Cardiovascular risk factors such as hypertension, hypercholesterolemia and diabetes, have been associated with a decrease in PPA.[37] Moreover, in patients with end-stage renal disease, a low PPA has been demonstrated to be a predictor for cardiovascular mortality.[38] Different classes of drugs display distinct effects on PPA: for example, many antihypertensive drugs (e.g. angiotensin-converting enzyme (ACE) inhibitors, calcium channel blockers (CCBs) and nitrates) increase PPA due to vasodilatory actions.[39] Developing tools to better predict PPA changes for both prognostic and safety reasons would help protect patient health by providing additional information to the systolic, diastolic and mean pressure values routinely measured.[32,39]

Furthermore, the shape of a BP trace is greatly affected by wave reflection.[32,40] The augmentation index (AIx) measures the pressure from the initiation of the reflected wave to the peak of the systolic curve, normalised to PP, and provides an indicator of wave reflection (Figure 6).[40] Although some studies have suggested that the relationship between

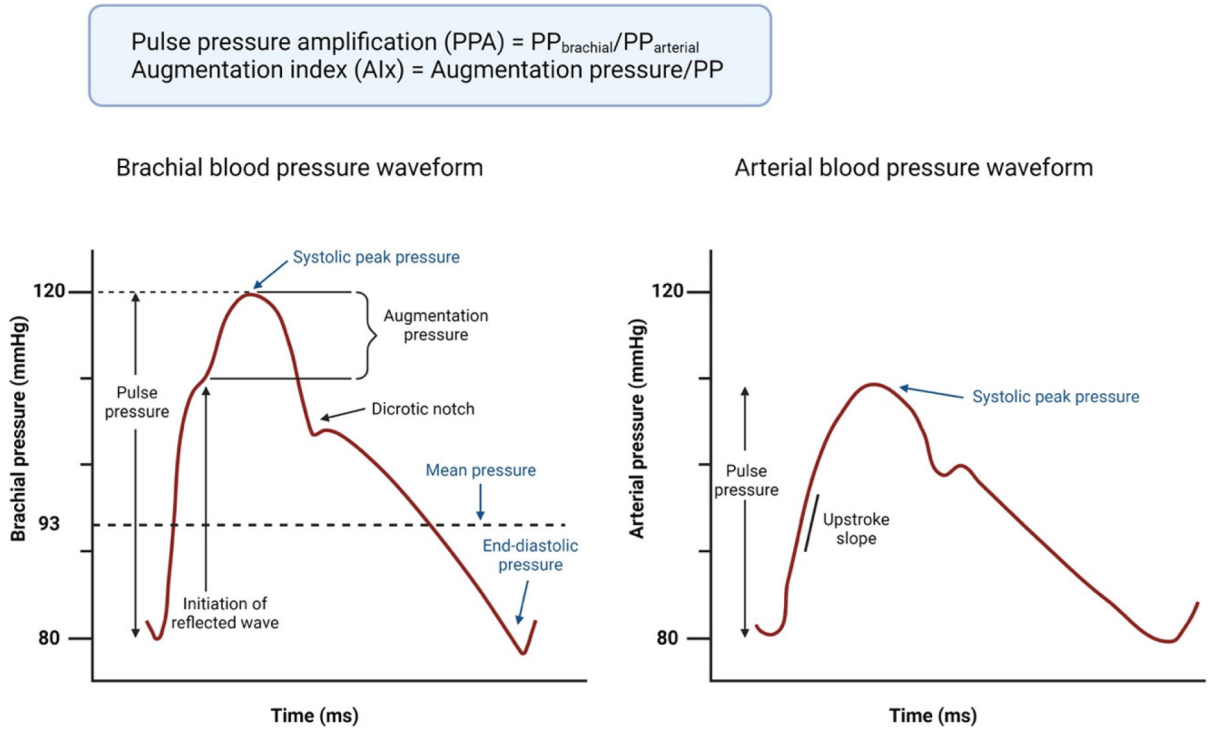


Figure 6. Analysis of waveform morphology changes. Pulse pressure (PP) and pulse pressure amplification (PPA), augmentation index (AIx), the position of the dicrotic notch and the slope of the upstroke segment can be used to assess changes in blood pressure waveform morphology. Created with BioRender.com.

the magnitude and effect are somewhat inconsistent,[41,42] it is apparent that as large arteries increase in stiffness, the reflected wave returns more quickly, with a resultant increase in pressure during LV ejection.[32,37] Together, this increases the likelihood of developing cardiovascular complications, including stroke, hypertension, diabetes and coronary artery disease.[37] An increased AIx is associated with an increased risk of cardiovascular events and pharmacological interventions resulting in a reduction of AIx are beneficial.[32]

Other parameters used in BP waveform analysis may include the upstroke slope, associated with LV contraction, and the temporal position and amplitude of dicrotic notch, mostly affected by closing of the aortic valve in the arterial waveform and the result of multiple reflected waveforms in pressures recorded peripherally (Figure 6).[35,43]

The examples of waveform analysis described above are mainly focussing on BP signals obtained from clinical settings. Nonetheless, the same concepts can be applied in the preclinical context (e.g. aortic/tail PPA in rats.[44]) Indeed, when evaluating cardiovascular safety liabilities *in vivo*, looking at minimal and maximal values of BP data alone may have value, but a more in-depth analysis of its waveform morphology and variability could potentially improve the translational power of these small laboratory animal models. Similarly, waveform analysis of Doppler flowmetry data could increase the sensitivity of detecting pharmacological or toxicological effects on the vasculature.

Conclusion

Cardiovascular toxicity remains one of the primary reasons for drug attrition in all stages of drug development,[1] highlighting the need for improved *in vivo* models.[2,7] Major challenges are comprehending the mechanism of toxicity and translating preclinical data to long-term outcomes in clinical settings.[2] The current gold standard for preclinical *in vivo* assessment of haemodynamics is radiotelemetry. This approach is a robust method to detect acute side effects and gives direction for clinical studies,[15,16] but is mostly limited to monitoring BP and HR.[15,18] In contrast, Doppler flowmetry is able to detect changes in blood flow in different vascular beds.[14,24] This model is, however, not widely used in the industry due to a lack of cost-effectiveness. In the current analysis of pressure and flow data, only averages or peak values are taken, disregarding most of the high-fidelity waveform data. More detailed analysis of the haemodynamic recordings may contain extensive information on the response of the heart and vasculature upon drug exposure. These additional insights could potentially expand the capability to precisely assess the risk of drug candidates early in their development and reduce the gaps that are currently present in preclinical safety pharmacology.

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