Validation of a new non-invasive blood pressure measurement method on mice via pulse wave propagation time measurement on a cuff

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Abstract

In the present article, we describe the validation of a new non-invasive method for measuring blood pressure (BP) which also enables to determine the three BP values: systolic, diastolic and mean value. Our method is based on the pulse transit time (PTT) measurement along an artery directly at the BP cuff. The accuracy of this method was evaluated by comparison with the direct simultaneous measurement of blood pressure from 40 anesthetized female mice. Close correlation between the gained data from these two methods was observed.

Keywords: diastolic pressure; direct blood pressure; mean pressure; non-invasive blood pressure measurement; pulse transit time; systolic arterial pressure.

Introduction

Mice as laboratory test animals play a significant and vital role in the medical research conducted. Therefore, the physiological parameters of the mice, BP and heart rate, need to be characterized.

The BP can be measured in an invasive or non-invasive way. The non-invasive way measurement is substantially more suitable than the invasive method for investigations where there is a mortality risk and possible health complications, and the life of the test animal must be preserved. Various methods of indirect measurement of arterial blood pressure in mice have been described [2, 3, 7, 14, 16]. In most of these methods, blood pulse or blood volume variations corresponding to the systolic end point are detected. These methods measure exclusively the systolic pressure. Other methods, based on the vascular unloading (Penáz method) technique [16] and the volume-pressure recording [4], are more suitable to measure the blood pressure in a continuous way. Although some studies have suggested that there is reasonable accuracy with these methods, the technology itself is generally expensive.

An alternative for monitoring the blood pressure with the passive non-invasive method is available with PTT measurements. However, this technique has found limited use due to inherent drawbacks in the existing technology. The electrocardiogram R wave is often used as a proximal timing point since it is simple to detect and tolerant of motion artifact. However, there is a considerable delay between the onset of electrical cardiac activity and the start of mechanical ventricular ejection. It is referred to as the pre-ejection period. Late studies, however, demonstrate that the use of electrocardiogram R wave purely for the assessment of the PTT is inappropriate and should be avoided since the pre-ejection period cannot be assumed to remain constant [13].

In this study, we describe a novel blood pressure monitor based on pulse wave velocity. This monitor consists of two photoplethysmography sensors in line. The second photoplethysmography sensor eliminates the need for the electrocardiogram and allows defining in just one procedure the three main features of the BP signals: systolic (SBP), mean (MBP) and diastolic (DBP). The importance of the three measurements is that the systolic and diastolic pressure represents the healthy state of the heart and the arteries, while the mean pressure shows the physiological characteristics of the left ventricular contractility, vascular resistance and elasticity averaged over time [6].

Methods

Subjects and materials

Measurements were conducted with a total of 20 female Naval Medical Research Institute (NMRI) and Fibroblast

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Figure 1 Schematic diagram for indirect measurement of mouse blood pressure.

Growth Factor (FGF) mice. A heterogeneous selection of mice (different age, weight and genealogy) was taken into account in order to validate the new method without requiring drugs for a specific increase or reduction of BP [8–10].

Figure 1 illustrates a schematic diagram of the device. The system consists of two photodiodes separated a minimum 15 mm from each other. This distance is based on the minimum detectable PTT of our system (3 ms) and the maximal pulse wave velocity reported in mice (4.60 m/s) [5]. They record the reflected light of an infrared LED attached to the tail of the mice. At the same time, a pressure cuff is initially inflated to a pressure (180 mm Hg) that exceeds the systolic arterial pressure of the mice. It is then reduced by a rate of approximately 2.5 mm Hg/s.

The recorded signals of the photodiodes are then used to estimate the PTT curve, this curve will be later correlated with the recorded signal of the cuff pressure. The method described in this paper can also be implemented in a trans-

mission mode if the distance between the light source and the sensors is smaller than 1 cm.

Principle

The result of the external pressure applied to the artery by the cuff is the modulation of the artery characteristics such as the compliance. This modulation can be described through the formula (1) [11, 15].

$$C(P_i, P_{cuff}) = \frac{R_{max}^2}{P_1} \left(\frac{1}{1 + \left(\frac{P_i - P_{cuff}}{P_1} \right)^2} \right) \quad (1)$$

P_1 : relates to the slope at the inflection point,

P_i : internal pressure, P_{cuff} = cuff pressure,

R_{max} : is the maximal asymptotic vessel radius.

Based on the Bramwell-Hill formula (2) can be demonstrated the relation between the PTT, the compliance, and cuff pressure [1].

$$v = \frac{d}{PTT} = \sqrt{\frac{1}{\rho C(P_i, P_{cuff})}} \quad (2)$$

ρ , blood density; d , distance between sensors.

Figure 2 shows the relation between the PTT and compliance. The three BP values are indicated.

The method used in this study takes advantage of this effect in order to estimate the BP indirectly. The PTT is calculated between the recorded signals of the photodiodes. It is obtained from the sensor nearest to the heart (proximal) and the signal of the sensor situated further away from the heart (distal). It shows the three characteristic features for

Figure 2 Modulation of the artery characteristics: compliance and PTT as function of the internal and the cuff pressure. It shows too the three BP values.

Figure 3 Characteristic points in the PTT curve. The descending curve shows the cuff pressure.

the determination of the systolic, the mean and the diastolic BP (Figure 3). The following paragraph explains the method developed on which we based this study. There are four phases:

- The cuff is initially inflated to a pressure of 180 mm Hg that exceeds the systolic arterial pressure of the mouse. As a result of the external pressure, the artery is totally compressed and therefore the blood cannot circulate and thus the pulse cannot be detected. The cuff pressure is reduced by a rate of approximately 2.5 mm Hg/s.
- At the time at which the cuff pressure is the same as the systolic BP, the blood can circulate again and the wave appears distal of the cuff, the transit time is at its minimum point. The increase of the transit time curve is considered a characteristic feature.
- Maximum of the transit time curve. At this point, the cuff pressure is equal to the mean BP. Due to this pressure; the compliance of the artery is maximal. Hence, the pulse wave velocity is minimal while the PTT is maximal [17].
- Once the compliance reaches the maximal point it starts to decrease and with it the PTT. When the cuff pressure is the same as the diastolic value, the modulation of the artery by the cuff is completed and the compliance remains constant. The buckling in the PTT curve is the diastolic BP.

Experiments with the mice

The measurements were approved by the District Governmental Commission on the Care and Use of Animals. The mice were anesthetized with Ketamin/Xylazin (120 mg/16 mg per kg weight) intravenously. The arterial carotid pressure was measured directly using a Samba micro-pressure sensor (SE-421 30, Samba Sensors AB, Västra Frölunda, Sweden) and an indwelling arterial catheter (Polyethylene-Catheter O.D.: 0.61 mm, I.D.: 0.28 mm, Becton-Dickinson, Heidelberg, Germany) that was carefully introduced, at maximum 0.5 cm into the vessel, in order to prevent entering the heart region. With the help of a trocar, the catheter became subcutaneously tunnelled directly to the neck area. The resigning end of the catheter was wrapped in fabric tape and was fixed with the help of a purse-string suture (Ethibond, 6-0, Ethicon). The transaction in the neck region was sewn in (Ethibond, 6-0, Ethicon). To determine the normalized

Figure 4 Pressure attenuation of the catheter (150 mm) in dependence of the frequency.

damping (D) of the pressure pulse obtained by the catheter-transducer system as a function of the frequency, a first pressure sensor (Medex Transducer, Smiths Medical, London, UK) was connected directly to the pressure generator in order to register the pressure practically without attenuation. A second sensor the Samba – micro pressure sensor was connected via a catheter to the pressure-generator, the pressure generator performed a frequency sweep between 0 and 20 Hz. The frequency dependence (Figure 4) can be described by the following formula:

$$D(f) = a \cdot e^{b \cdot f} + c \cdot e^{d \cdot f} \quad (3)$$

with $a = 1.6640$, $b = -0.3331$, $c = 0.4582$ and $d = -0.0641$.

The formula was generated with the Matlab's curve fitting tool by which the constants a , b , c , d are determined.

Hence, it is necessary to implement a correction factor in order to compare the direct and indirect measurements. For this reason the heart rate was estimated using the first harmonic frequency from the fast Fourier transform analysis of the proximal photodiode signal and this value is used to calculate the damping. To compensate the damping is used in the following formula:

$$direct\ BP_{compensated} = \frac{direct\ BP_{measured}}{D(heart\ rate)} \quad (4)$$

This compensation was made only for the pulsatile components of the direct BP measurements (DBP and SBP) and not for the steady components (MBP), because the catheter damping in the range of the steady components does not affect the measurements as can be seen in the Figure 3.

During the operation and the recovery phase, the body temperature of the animals remained constantly at 36°C using special heating plates. The first measurement of the direct BP and the PTT were performed at the same time after the mice were woken up and after a relaxation time of at least 2 h post operation. The mice were placed in a rodent holder (Mouse rodent holder Model 84, IITC, California) so that the catheter could be easily accessed from outside.

Once the photodiode signals and the cuff pressure signal were recorded, the pulses of the proximal and distal signals were detected. Each pulse was found by the identification of the maximal positive slope points of the signals. The PTT curve was calculated as the time difference between the corresponding detected points of the proximal and distal signals.

Results

6 ± 2 measurements were carried out per mouse. Figure 5 shows a typical change of the transit time compared to a simultaneous direct BP measurement during the deflation phase of the cuff. The calculation of the indirect BP is based on the beat to beat measurement of the transit time and the locations of the three characteristic features: systolic, diastolic and mean blood pressures.

Figure 6 shows an example of the recordings of the proximal and distal signals (A), the PTT curve as a result of the time difference between both signals with the corresponding BPs (B) and the cuff pressure (C).

In Figure 7, the data of systolic, diastolic and mean blood pressures are summarized using a regression method (5B, 5D, 5F) and the Bland-Altman plot (5A, 5C, 5E) which is a statistical method to compare two measuring techniques. In the Bland-Altman plot, mean and standard deviation are calculated for the present system vs. the direct method. Table 1

Figure 5 Typical PTT alterations (x) during a deflation phase of the cuff (X-axis) compared to the direct measurements (right Y-axis). The vertical lines show the BP determined by the interpolation with the cuff pressure. At the 100 mm Hg cuff pressure the blood could circulate and the transit time is at its minimum point, it is the systolic pressure. At the 80 mm Hg cuff pressure, the pressure is equal to the mean pressure and the PTT curve has a maximum. At the 60 mm Hg cuff pressure the PTT curve has a buckling and it is the diastolic pressure.

Figure 6 An example of the recorded distal and proximal signals (A). PTT curve with the SBP, BMP and DBP points (B). Cuff pressure curve with the corresponding BP points (C).

summarizes the comparison between the direct and the indirect measurements.

Value differences can be explained by the fact that there is a certain significant distance between the measuring positioning of the sensors and the heart. Therefore, the indirect systolic BP values are higher and the mean and diastolic BP values are lower than those of the direct measurements.

In order to be able to compare our measuring values to the values described in the literature, the average BP values from several measuring cycles of a session were calculated.

With this procedure, the comparison of the blood pressure-value-pairs is more related to the mice than to the measuring cycles. Table 2 summarizes the comparison between the direct and the indirect measurements.

Discussion

In comparison to other methods, the novel indirect BP measuring method is at the moment the only non-invasive intermittent method which delivers three discrete characteristics for the identification of systolic, diastolic and mean blood pressures. Furthermore, this BP measuring method is based on a BP cuff which measures the BP intermittently.

The determination of the PTT along an arterial vessel directly at the BP cuff offers another big advantage: due to the physiology of the vessel, the cuff pressure dependent measurement of the transit time delivers three discrete characteristics to the determination of the systolic, mean and diastolic BP. Up until now this phenomenon has not been described in the literature.

The comparison of the two BP measurements, applying the Bland-Altman statistical method, shows that the results of the new BP measuring method agree with the directly measured BP. The relatively small standard-deviation of the diastolic and mean BP difference indicates that the charac-

Figure 7 Indirect against indirect measurements: Systolic (SBP) (A, B), mean (MBP) (C, D), diastolic (DBP) (E, F) BP comparison to method of Bland and Altman (left) and to regression method (right) of all BP value-pairs bet. R, regression-coefficient; MW, blood pressure-difference; SD, standard-deviation.

Table 1 Statistical comparison between direct and indirect BP measurements (related to measuring cycles).

BP	Difference		p-Value	Regression coefficient
	Mean mm Hg	SD mm Hg		
SBP	5.32	14.38	0	0.71
MBP	-0.87	13.19	0	0.76
DBP	0.35	12.73	0	0.78

Table 2 Statistical comparison between direct and indirect BP measurements (related to mice).

BP	Difference		p-Value	Regression coefficient
	Mean mm Hg	SD mm Hg		
SBP	4.81	10.98	0	0.80
MBP	1.22	9.30	0	0.85
DBP	0.02	8.00	0	0.89

teristics of the PTT curve are continuously stable. On the other hand, the comparisons of the systolic BP measuring values show a lower stability.

Measuring the cuff pressure by the systolic BP does not provoke a clear change in the transit time curve in some animals. As a result, the transit time gradient does not show a distinctive onset in each animal.

The most important factor influencing the accuracy of the measurement relies is the calculation of the PTT. It implies the pulse recognition in the distal and proximal photodiode signals and the time difference between the corresponding detected pulses. Taking into account that the maximal pulse velocity is 4.60 m/s, for short distances between sensors, the PTT has a magnitude of milliseconds. In order to improve the accuracy of the method it is recommended to increase the signal-to-noise ratio of the proximal and distal signals.

In conclusion, the proposed method has shown to be a useful tool for the indirect measurement of the arterial pressure in the mouse tail. In pursuance of the Langewouters study [12] there is similarities between the anatomy of the mouse tail and the human finger. With this premise, the proposed method seems to have a good perspective to find use in applications for humans.

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