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In vivo detection of venous sinus distension due to intracranial hypotension in small animal using pulsed-laser-diode photoacoustic tomography

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KEYWORDS: cerebrospinal fluid, intracranial hypotension, pulsed laser diode, photoacoustic tomography, venous sinus distension

Abstract

Intracranial hypotension (IH) is a pathophysiological condition of reduced intracranial pressure caused by low cerebrospinal fluid (CSF) volume due to dural injuries like lumbar puncture, surgery etc. **Understanding the prognosis of IH in small animal models is important to gain insights on the complications associated with it such as orthostatic headache, cerebral venous thrombosis, coma, etc. Photoacoustic tomography (PAT) offers a novel and cost-effective way to perceive and detect IH in small animal models.** In this study, a pulsed laser diode (PLD) based PAT imaging system was used to examine the changes in the venous sinuses of the rat brain due to IH, induced through CSF extraction. **After the CSF extraction an increase in the sagittal sinus area by ~30% and width by 40±5% was observed. These results provides**

supportive evidence that the PLD-PAT can be employed for detecting changes in sagittal sinus due to IH in rat model.

Abbreviations: [IH, intracranial hypotension; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; PA, photoacoustic; PAT, photoacoustic tomography; PLD, pulsed laser diode; UST, ultrasound transducers; SUTR, single-element ultrasound transducer; CVT, cerebral venous thrombosis; TS, transverse sinus; SS, sagittal sinus]

1 INTRODUCTION

Intracranial hypotension (IH) is a pathophysiological condition of reduced intracranial pressure caused by the spontaneous leak of CSF due to dural injuries such as lumbar puncture, trauma, or injury [1]. Since its description by German neurologist Georg Schaltenbrand in 1938, IH has been widely recognized as the cause of persistent headaches and its misdiagnosis is typical [2-4]. The common complications associated with IH are acute orthostatic headache, gait disturbance, movement disorder, hyperacusis, diplopia, memory deficits, and photophobia [5, 6]. Apart from these, rare but serious complications of untreated IH include subdural hematoma, subarachnoid hemorrhage, cerebral venous thrombosis (CVT), cranial nerve palsies, sagging of brain stem, small asymptomatic aneurysms, and even coma [7-9]. As IH can be debilitating, a cost effective means to understand it is intrinsic. Conventionally magnetic resonance imaging (MRI) has been the preferred choice of imaging to diagnose and understand the prognosis of

IH [5]. However, MRI has limitations like longer imaging time, low temporal resolution, and requirement of contrast agents for detecting IH. Also, MRI is very expensive.

Photoacoustic Tomography (PAT) is an emerging noninvasive hybrid imaging modality that combines the merits of optical (high absorption contrast) and ultrasound imaging (high spatial resolution) [10-14]. Over the last decade, PAT has shown many potential applications in clinical and preclinical studies [15-22]. In PAT, the nanosecond laser pulses irradiating the biological tissues generate ultrasound waves [attributed as photoacoustic (PA) waves] due to light absorption and thermoelastic expansion by the chromophores. These PA waves are then acquired at various locations around the tissues by employing broadband ultrasound transducers (UST). The collected signals are then used to reconstruct the cross-sectional PAT image by mapping initial pressure rise distribution using various reconstruction algorithms [23, 24].

In recent years, pulsed laser diode (PLD) has been gaining attention as an excitation source for PAT imaging systems. This is due to its lower cost, compact size, and high repetition rate in comparison with the traditional Nd:YAG based laser excitation sources [25-32]. PLD based PAT system for various applications such as brain imaging, monitoring wash in and wash out of contrast agents, diagnosis of cardiovascular diseases, and diagnosis of rheumatological disorders have been demonstrated already [33-35]. Use of acoustic reflector based single-element ultrasound transducer (SUTR) instead of the traditionally used horizontal UST's makes the PLD-PAT system even more compact by reducing the scanning radius [36]. In this study, a desktop PLD-PAT imaging system was used to detect and monitor the intracranial hypotension induced in rats through cerebrospinal fluid (CSF) extraction. The morphological changes in the cerebral venous sinuses of the brain due to IH were assessed using PLD-PAT imaging. These results confirm that PAT posits a great potential to detect the progression of wide spectrum of

intracranial pressure related pathologies such as CVT, hydrocephalus, etc. in preclinical animal models.

2 MATERIALS AND METHODS

2.1 PLD-PAT imaging system

The schematic of the desktop PLD-PAT imaging system is shown in **Figure 1(a)**. A ~816 nm wavelength PLD (Quantel, QD-Q1924-ILO-WATER) capable of delivering 2000 pulses per second at ~107 ns pulse width was used to image the animal. The PLD was controlled by a laser driving unit consisting of a water-cooling unit, a low voltage power supply of 12V (Votcraft, PPS-11810), a variable high voltage power supply (Elektro-Automatik, EA-PS 8160-04 T) to control the power of the laser, and a function generator (RIGOL, DG1022) to control the pulse repetition rate. The laser beam was homogenized by an optical diffuser. The homogenized laser beam had a power of 6.8 W and a pulse repetition rate of 2 kHz. The laser beam is expanded over the area of ~20 cm² for irradiation and the fluence was maintained at ~0.17 mJ/cm² per pulse on the sample surface, which is at par with the American National Standards Institute (ANSI) safety limit for a scan time of 12 s [37]. The laser beam The fluence of the laser beam can be reduced by decreasing the laser power or reducing the pulse repetition rate. The generated PA waves were acquired by an unfocused UST (Olympus-NDT, V309-SU) of 5 MHz center frequency (13 mm active area, 70% fractional bandwidth), compounded with a 45° acoustic reflector (F102, Olympus NDT). The PA waves were collected by continuously rotating the SUTR 360° around the animal brain using a stepper motor controlled by the computer. The PA signals were amplified by a 48 dB low signal noise amplifier (Mini-circuits, ZFL-500LN-BNC), and then stored inside a computer (Intel Xeon, 3.7 GHz 64-bit processor, 16 GB RAM) using a data acquisition (DAQ) card (Spectrum, M2i.4932-Exp) synchronized with the laser excitation using the function generator.

2.2 Animal preparation

For the in vivo study, seven NTac: Sprague Dawley®SD® female rats (weighing $\sim 90 \pm 5$ gms) procured from InVivos Pte. Ltd, Singapore were utilized. The rats were divided into two groups: control group ($n = 6$) and CSF group ($n = 6$). Experiments were carried out with one rat at a time and the steps mentioned below were similar for all of them. Each rat was anesthetized before the imaging. The induction of anesthesia was achieved with a combination of Ketamine (100 mg/ml) and Xylazine (20 mg/ml). Before imaging, eye gel was applied on both the eyes, hair on the rat head was removed by depilatory cream, and a layer of ultrasound gel was applied for better ultrasound coupling. The photographs of the rat head with hair, and without hair are shown in **Figures 1(b) and 1(c)**, respectively. For imaging, the rat was placed in an upright position, on its abdomen, using a customized holder, and its body was secured using surgical tape. During the entire course of the experiments, a continuous inhalation of 0.75% isoflurane and 1.0 L/min oxygen were provided for maintaining the anesthesia. A translational stage was used to position the rat head on the imaging plane. After the experiments, the rat was euthanized by administering Valbarb (sodium pentobarbitone 300 mg/ml) intraperitoneally. The rat head with scalp open is shown in **Figure 1(d)** for demonstration purpose only. All PA imaging and analysis was done on rats with intact scalp [**Figure 1(c)**].

2.3 Induction of intracranial hypotension

Intracranial hypotension (IH) is an established medical phenomenon caused due to the depletion of CSF, as CSF pressure is the modulating factor for intracranial pressure [38, 39]. For inducing the IH, the CSF leak was mimicked by extracting the CSF from the cisterna magna, the most easily accessible subarachnoid space opening in adult rats. A CSF volume of $\sim 45 \pm 5$ μ l was extracted using a needle connected to a draw syringe by positioning the rat head at an angle of 45° . CSF extraction without the contamination of blood was ensured. The CSF extraction

procedure was followed for all the rats in the CSF group, whereas no CSF extraction was performed on the control group.

2.4 Photoacoustic brain imaging

In vivo brain PA imaging was performed using the PLD-PAT system by scanning the SUTR 360° around the rat head for 12 seconds. Although the desktop PLD-PAT system is capable of imaging in duration as short as 4 seconds, 12 seconds imaging duration was used to obtain a better signal to noise ratio, considering the observational time scale (minutes) of changes in the brain due to IH. For the rats in the control group, 19 brain images at an interval of 10 minutes were obtained and for the rats in the CSF group, 8 brain images (baseline images) were obtained before the CSF extraction and 37 brain images were obtained after the CSF extraction at an interval of 4 minutes each. The total duration of the experiments was ~180 minutes. The cross-sectional PAT image of the brain was reconstructed from the acquired PA signal using a simple delay-and-sum algorithm [40, 41].

3. RESULTS AND DISCUSSION

The PA images of a rat brain in the control group (**one representative**) are shown in the **Figures 2(a-c)** at three different time points. The PA images of a representative rat brain in the CSF group before the CSF extraction, 5 minutes post CSF extraction and 149 minutes post CSF extraction are shown in **Figures. 2(d-f)**, respectively. A characteristic change that occurs in the brain to maintain homeostasis following the spontaneous loss of CSF is cerebral venous sinuses distension [42, 43]. The cerebral venous sinus distension provides us a reliable diagnostic clue to interpret IH [44]. But in conventional imaging modalities such as MRI, CT, etc. this phenomenon is not easily quantifiable, and misdiagnosis is a common occurrence [45]. From the pre and post CSF extraction PAT brain images of the CSF group, the changes in the contour of the cerebral venous sinuses can be prominently noticed [**Figures 2(e, f)**]. **The PAT brain**

images of all the rats in the CSF and control group before and after the CSF extraction point are shown in the Fig. S1 and Fig. S2 of Supporting Information. Please note that the SS area of a rat varies according to their head size and weight. Therefore, one cannot compare the SS size among different animals. Instead the comparison needs to be done before and after intracranial hypotension happens for the same animal.

To understand the results better, the areas of the SS region on the brain images of both the CSF and control group were calculated. The SS was chosen for the evaluation of the area due to its higher sensitivity, specificity, and diagnostic accuracy towards IH in comparison with the two transverse sinuses (TS) [46]. To calculate the SS area, the reconstructed PA brain images were first contrast enhanced using histogram equalization method and then binarized with 50% of the maximum value as threshold. Next the region of interest (SS) was separated from the background. The number of pixels with the binary value 1 in the region of interest was calculated and multiplied with the area of an individual pixel (0.625 mm^2) to obtain the actual SS area. The block diagram representing the steps for calculation of SS is shown in **Figure 3**. All image processing was done using MATLAB. The calculated SS areas of each rats in both the CSF group and control group were normalized by dividing the SS areas calculated at each time points with the initial SS area.

Figure 4(a) shows a plot of the normalized SS areas of one **representative** rat each from the control group (black circles) and the CSF group (red square). It can be noticed from the plot, that the mean SS area of the rat in the control group is almost similar throughout the entire duration of the experiment due to the absence of CSF extraction procedure. Whereas, in the case of the rat from the CSF group there is a marked increase in the SS area after CSF extraction, which is in correlation with visually noticed cerebral venous sinus dilation on the post CSF extraction brain images of the CSF group. Even though slight variations were noticed, the

change in the sinus area due to CSF extraction persisted over 150 minutes post CSF extraction. The slightly increasing trend in the SS area of the control group rat was because of the water pressure on the rat head and isoflurane exposure over a long duration. **The plot of the normalized SS areas of all the rats in both control and CSF group are shown in the Fig. S3 of Supporting Information.**

Statistical analysis was performed on the SS areas of all rats in the control and CSF group over the interval demarcated by the dotted box in **Figure 4**. The mean value of the SS area obtained from the control group was 1.02 and over the pre-CSF extraction period of the CSF group was 1.04. After the CSF extraction, the mean SS area over the post CSF extraction period of the CSF group increased to **1.4**. The increase in the area is due to the occurrence of intracranial hypotension caused by the plummet in CSF volume. **Figure 4(b)** shows the bar graph of the mean SS areas with standard deviation for the control group and the CSF group (pre and post CSF extraction). The percentage difference was calculated between the SS areas of the pre-CSF extraction period and of the post-CSF extraction period of the CSF group. It was found that the area of the SS increased spontaneously by ~33% after the CSF extraction (paired t-test, p-value <0.001). p-value <0.05 was the criterion for statistical significance.

The line profiles across the SS at a similar position [red dotted line in **Figures 2(d-f)**] are shown in the **Figures 5(a-c)**. The line profiles were normalized between the range 0 and 1 to calculate their respective full width half maximum (FWHM). The calculated FWHM values indicate that the width of the SS increased from 493 μm (before CSF extraction) to 677 μm (5 minutes post CSF extraction) and 705 μm (149 minutes post CSF extraction). **Also, line profiles and the respective FWHM from the images before CSF extraction, 5 minutes post CSF extraction and 149 minutes for all the other animals in the CSF group was calculated (please refer to Fig. S4**

of the supporting information). The statistical analysis suggests that the width of SS increases by $40\pm 5\%$ after the CSF extraction.

In practical scenarios, IH most commonly occurs after the medical or surgical interventions in the spine and is often misdiagnosed. Also, the cost associated with the diagnosis of IH is high. In such cases, the correlation of the PA imaging data obtained before and after the intervention with the associated symptoms can lead to accurate, low cost diagnosis of IH. There are few other scenario, where there will be no pre-hypotension data available. In such cases, cut off levels must be formulated from population-based studies, as it is the case with other medical protocols and modalities. For example, in the case of spinal canal stenosis due to degenerative disc disease of the spine, the absolute spinal canal diameter is determined by MRI, but whether it is sufficiently low to warrant surgical intervention depends upon the guidelines set after population-based trials and corroborative clinical evidence. Although, in current scenario application of PAT for clinical brain imaging is challenging due to the light and sound attenuation by the skull. Numerous studies suggests that the PAT can be possibly translated to clinical brain imaging by optimizing incident light delivery, employing ultrasonic detection at frequencies lower than 1 MHz (higher frequencies sound waves are attenuated more by the human skull than low frequency signal) [47], employing improved reconstruction methods to correct acoustic signal aberrations (scattering and absorption) [48], or by using contrast agents to enhance the signal to noise ratio (SNR) [49]. The lower energy of the PLD laser excitation source is also a limitation in the translation of PLD-PAT for clinical imaging at its current form. However, it is conceivable that the higher energy PLD lasers suitable for clinical imaging will be available in the future due to the rapidly evolving laser technology. Our study is a preliminary one which proves the capability of the PLD based PAT system to detect this pathophysiological change in the rat brain.

The temporal resolution of the PLD PAT system can be significantly improved to visualize the real-time blood flow dynamics and morphological changes in the venous sinuses due to IH by employing multiple SUTR's. Spectroscopic capabilities can also be added to the PLD-PAT system by employing a multi-wavelength PLD. The findings of our pilot study demonstrate the potential of PLD-PAT imaging system to diagnose IH in **preclinical animal models**. Furthermore, the PLD-PAT system can be applied towards the diagnosis of various pathologies, which has contour changes of cerebral venous sinus associated with it such as CVT, hydrocephalus, etc. This will lead to a development of a highly accurate, low cost, and rapid imaging system to diagnose the **progression of IH and related pathologies in preclinical models**.

4. CONCLUSION

In this study, the potential of the PLD-PAT system to noninvasively detect **and monitor** the IH by evaluating the morphological changes in the cerebral venous sinuses of the brain was demonstrated using a preclinical rat model. The induction of IH was performed by extracting the CSF from the cisterna magna of the rats. PAT imaging was performed before and after the induction of IH to monitor the changes in the brain due to IH. These results and analysis demonstrate that the contour change in the cerebral venous sinuses, which is a reliable sign for the detection of IH [44], can be imaged with high specificity using the noninvasive PLD-PAT imaging system. More than 30% change in the SS area was observed with photoacoustic imaging before and after the IH occurrence.

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AUTHOR CONTRIBUTIONS

P.R., S.S., M.P. conceived and designed the study. P.R. performed all the experiments, data acquisition, and analysis of the data. S.S. did the interpretation of the data and the extraction of CSF. R.A.D. prepared the animals for all the experiments. M.P. supervised the study. All authors contributed to the writing of the manuscript.

CONFLICT OF INTEREST

Authors have no relevant financial interest in this work and no other potential conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Figure S1: In vivo PAT brain images of all the rats in the control group before and after the CSF extraction point. Herein, SS, sagittal sinus; TS, transverse sinus; CV, cerebral veins.

Figure S2: In vivo PAT brain images of all the rats in the CSF group before and after the CSF extraction point. Herein, SS, sagittal sinus; TS, transverse sinus; CV, cerebral veins.

Figure S3: Normalized sagittal sinus (SS) areas of all the rats in both the control group and the CSF group over the entire duration of the experiment. The red arrow indicates the point of CSF extraction and the dotted region indicates the interval used for statistical analysis.

Figure S4: line profiles across the SS at similar position obtained from the images before CSF extraction, 5 minutes post CSF extraction and 149 minutes post CSF extraction for all the rats in the CSF group. The overall increase in the width of SS after the extraction of CSF is $40 \pm 5\%$.

FIGURES:

FIGURE 1. (a) Schematic of the PLD-PAT system for in vivo detection of intracranial hypotension: SUTR-single-element ultrasound transducer with reflector; AMP-amplifier; SM-stepper motor; PLD-pulsed laser diode; LDU-laser driving unit; AM-anesthesia machine; PC-personal computer; DAQ-data acquisition card. Photograph of the rat head (b) with hair intact, (c) hair removed before PA imaging, (d) scalp open after the animal was euthanized (for demonstration purpose only).

FIGURE 2. In vivo brain PAT images of the rats using PLD-PAT system: images of a rat in the control group at (a) -28 minutes, (b) 10 minutes and (c) 150 minutes. Brain images of a rat in the CSF group (d) before CSF extraction (-28 minutes), (e) 5 minutes, and (f) 149 minutes post CSF extraction. Herein, SS, sagittal sinus; TS, transverse sinus; CV, cerebral veins.

FIGURE 3. Block diagram for the calculation of sagittal sinus area from the cross-sectional PAT reconstructed images of the brain.

FIGURE 4. (a) Normalized sagittal sinus (SS) areas of one rat each from the control group and the CSF group over the entire duration of the experiment. The red arrow indicates the point of CSF extraction and the dotted region indicates the interval used for statistical analysis. (b) Mean sinus area of the control group and CSF group (pre-CSF extraction, post-CSF extraction) with standard deviation.

FIGURE 5. (a-c) Three-line profiles across the SS at a similar position [corresponding to the red dotted line in Figures 2(d-f)].

Graphical Abstract

Intracranial hypotension (IH) is a condition of reduced intracranial pressure, its timely diagnosis is important to avoid the associated complications. Photoacoustic tomography, offers a novel and cost-effective way to detect IH. This study demonstrates the potential of pulsed laser diode based photoacoustic tomography imaging system to diagnose intracranial hypotension using a preclinical animal model.