Assessment of liver function reserve by photoacoustic tomography: a feasibility study

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Abstract: Assessment of liver function reserve (LFR) is essential to determine liver resection scope and predict prognosis for patients with liver disease. Indocyanine green (ICG) concentration change is a classic marker to reflect liver function reserve as ICG is selectively taken up and eliminated by liver. Here we proposed a noninvasive approach for LFR assessment based on a real-time photoacoustic tomography (PAT) system. This feasibility study was to detect ICG concentration change by PAT in phantom and in vivo using both normal and partial hepatectomy (PH) rabbits. A linear relationship between photoacoustic signal intensity of ICG and ICG concentration was found in vitro. In vivo ICG concentration change over time after ICG injection was observed by PAT in normal rabbits, which was consistent with the findings measured by invasive spectrophotometry. Finally, clear difference in ICG clearance between the control and PH models was identified by PAT. Taken together, our study indicated the clinical potential of PAT to in vivo evaluate LFR noninvasively.

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1. Introduction

Liver function reserve (LFR) is the remnant functional capacity of liver after liver injury. Preoperative LFR assessment was necessary and critical since patients with impaired LFR carries higher risk of posthepatectomy liver failure (PHLF) than normal liver resection [1–3]. PHLF is one of the most serious complications and the major cause of mortality (30%) after liver resection [4,5]. On the other hand, for patients with chronic or acute liver disease but no need for surgery, LFR assessment can help with determination of clinical treatment and prediction of prognosis [6].

Traditional method like Child-Pugh scoring system is widely used for LFR evaluation but it suffers from low sensitivity and specificity [7]. Imaging modalities such as liver volume measurement by computed tomography (CT) plays an important role for setting surgery plan. However, this approach reflects LFR in a quantity rather than a quality perspective, thus it’s especially not accurate in patients with liver fibrosis or cirrhosis [8]. Gadodextrin acid-enhanced magnetic resonance imaging (MRI) and Technetium-99m diethylenetriaminepentaacetic acid galactosyl human serum albumin single photon emission computed tomography (99mTc-GSA SPECT) can provide deep penetration and multi-parameter information for LFR evaluation. However, both of them suffers from significant limitations. Gadodextrin acid-enhanced MRI is expensive and requires many individual breath-holds and a long data acquisition time, which makes imaging of dynamics difficult [9]. 99mTc-GSA SPECT is not routinely performed in most
centers and suffers from poor spatial resolution, ionizing radiation exposure and high economic cost as well [10].

Indocyanine green (ICG) clearance test is the most acknowledged way of assessing LFR. ICG is a water-soluble, FDA approved dye, which can be selectively taken up by liver cells, eliminated in the original form through bile without any metabolic change or extrahepatic intake [11]. Due to this exclusive hepatic clearance, ICG retention or elimination rate has been a widely used marker for LFR evaluation. The classic and gold standard approach for detecting ICG retention or elimination rate is spectrophotometry. However, it demands multiple blood sampling, which is an invasive, complex and not real-time procedure [3]. Another indirect detecting method is pulse dye densitometry (PDD), which is non-invasive. However, it’s a blind test without any imaging guidance and the accuracy of the result can be affected by multiple factors such as perivascular tissue, hemoglobin concentration, weak arterial pulse. Moreover, the testing principle of PDD is based on two-wavelength near-infrared light, the slight shake of tested part such as nose, finger, will lead to an inaccurate result [12]. Evidence has been provided for the poor consistency between PDD and the gold standard spectrophotometry [13].

Therefore, a non-invasive, accurate, convenient and economic method to perform ICG clearance test for LFR assessment is in demand. Can photoacoustic tomography (PAT), a fast-developing imaging technique make a difference? PAT captures ultrasound signals (photoacoustic signal, PAS) generated from tissue thermally expanded by laser pulses to reconstructs images [14]. Based on optical absorption, PAT can afford high resolution images in superficial or deep tissue. This novel non-invasive imaging modality has exhibited its utility in breast cancer [15], thyroid cancer [16], vascular examination [17], arthritis [18] as well as bowel disease [19]. On the other hand, PAT has been applied to quantify endogenous chromophores such as hemoglobin, melanin, lipids, collagen based on the unique optical absorption properties of these substance [20–23]. ICG is not only a drug for LFR reflection, but also a common exogeneous contrast agent for photoacoustic imaging due to its unique optical absorption spectrum. Previous studies validated the relationship between PAS of ICG and ICG concentration as confirmed by measurements by fluorescence imaging or spectrophotometry [24–27]. Studies aiming at direct visualization of various liver diseases using PAT imaging showed promising results in mouse models of fatty liver or liver fibrosis. By photoacoustic spectral analysis of liver, hepatic fat rate or fibrosis severity could be evaluated non-invasively [28–30]. However, due to penetration limit of PAI/PAS, these applications were realized only in small animals, and much work is needed for testing its potential for clinical translations. Furthermore, LFR assessment by PAI on peripheral vessel has not been considered in previous studies using mouse models of various liver diseases.

This study is for the first time to investigate PA-based LFR on central auricular artery in normal and partial hepatectomy rabbit models. PAT may become a promising and useful imaging tool for LFR assessment in a clinical setting.

2. Methods

2.1. Photoacoustic tomography system

The schematic description of PAT system is presented in Fig. 1. For more detail about this system can be found in previous study [17]. The illumination system contains a Q-switched Nd:YAG-pumped optical parameter oscillator system (Surelite, Continuum, California) capable of supporting pulses at wavelength from 700 to 960 nm with 4 ns pulse duration at a repetition rate up to 20 Hz. An optic fiber bundle with line shaped illumination profile (40 mm × 10 mm) was set besides the transducer to supply lighting. The incident fluence at 805 nm was estimated to be 5 mJ/cm², which is below the American National Standards Institute safety limit [31].

The imaging probe was made up of the optic fiber bundle and a 128-element concave ultrasound transducer array (Japan Probe Corporation, Yokohama, Japan). The diameter of the transducer
Fig. 1. A schematic of the experiment system. The hardware components employed in the PAT system. DAS: Data Acquisition System (preamplifiers, multiplexers and analog-to-digital converters). OPO: Q-switched Nd:YAG-pumped optical parameter oscillator system. PC: personal computer. Imaging Probe (red dotted box): a 128-element transducer array, a fiber bundle and a resin shell enclosed with a membrane.

was 100 mm and the central frequency was about 5 MHz (90% bandwidth). The probe was fully enclosed with a 100µm polydimethylsiloxane (PDMS) film and filled up with deionized water.

The PA pressure waves from the element were amplified through a custom-built pre-amplifier then transferred to a 64-channel analog-to-digital system (8 PXIe5105 cards, National Instrument, Texas, USA) after 2:1 multiplexing. The signal was sampled at 12-bit digital resolution and a sampling rate of 50 MS/s. The acquired signals were saved in the onboard computer (PXIe8840, National Instrument, Texas, USA) which also worked as control panels for the PAT system.

A commercial ultrasound platform (iNSIGHT 23R, Saset Healthcare, Inc. Chengdu, China) were used in our research to acquire ultrasound images with 7.5MHz line transducer array.

2.2. In vitro ICG concentration measurement by PAT

According to manufacturer’s instructions for standard curve making, concentrations of 0.1, 0.2, 0.3, 0.5, 1.0mg/dl ICG solutions were made. 1ml distilled water plus 1ml normal saline plus 1ml normal serum was mixed together as the blank control. The absorbance of each concentration and the blank control was measured by spectrophotometry (ZB006, Shanghai INESA Scientific Instrument Co., Ltd, China) at a wavelength of 805nm.

After spectrophotometry measurement, the photoacoustic signal intensity (PSI) of each concentration and the blank control was obtained by PAT. A half cylindrical phantom with 2cm diameter was used as the background. The optical parameters of the phantom referred to previous study [32]. ICG solution was encapsulated in a 3mm diameter transparent PVC pipe then embedded in the phantom.

2.3. In vivo ICG concentration measurement by PAT in normal rabbits

New Zealand rabbits (n = 7, male, weight: 2.5-2.7kg) were fed with food and water for a week of acclimatization period and then were anesthetized by isoflurane gas inhalation in the lab at a room temperature of 26 centigrade. The rabbit was in right lateral position. Its right ear was shaved and fixed on a flat plate (Fig. 2(a)) with the central auricular artery (CAA) clearly visible as shown in Fig. 2(b). The PAT imaging probe was placed above the right CAA and the ICG was injected through the left auricular vein. Transverse PAT and US sections of the artery was displayed as the region of interest (ROI) in Figs. 2(c) and 2(d), respectively (yellow dashed circle and arrow).
Fig. 2. In vivo imaging of rabbit ear. (a). Photograph of the photoacoustic imaging setup. (b) Photograph of rabbit ear under imaging showing the clearly visible central auricular artery (CAA) morphology and location. Transverse photoacoustic (c) and US (d) images of the rabbit ear with the CAA marked by yellow dashed circle and arrow, respectively. Region of interest (ROI) (yellow dashed circle) was selected to determine the photoacoustic signal intensity.

Before the PAT scan, ICG was dissolved in distilled water to obtain a concentration of 5 mg/ml. Two ml blood was collected through the left central auricular artery (CAA) via an anticoagulant vacuum tube. PAT scan at 805nm was performed for 20 minutes over the right CAA. The acquisition rate was 1 frame per second (Time-averaged for 10 consecutive frames). One minute after a baseline PAT scan, a dose of 0.1 ml/kg ICG solution was injected via the left auricular vein along with 5 ml saline flush for 10 seconds. Then the PAT signal was continuously acquired for additional 19 minutes.

At the same time, blood was collected every one minute (≥ 5 time points, 2ml blood for each time point) through left CAA into the anticoagulant vacuum tube. The removed blood volume was replaced by an equivalent volume of normal saline. Blood collected at different time points was centrifuged at 3000r/min for 10 minutes. Then the plasma was collected for spectrophotometry measurement and PAT imaging (in the same phantom described in previous part) in vitro respectively, both at wavelength of 805nm.

2.4. In vivo ICG concentration measurement by PAT in partial hepatectomy models

New Zealand rabbits (n=10, male, weight: 2.5-2.7kg) were fed with food and water for a week of acclimatization period and randomly assigned into partial hepatectomy (PH) group (n=4) and control group (n=6). Rabbits were anesthetized by isoflurane gas inhalation in the lab at a room temperature of 26 centigrade. PH surgery was performed under sterile conditions for the PH group. Xiphoid process was exposed by a midline laparotomy incision. The left lateral lobe, left and right median lobe were ligated at the base by 4/0 silk ties and removed. The volume of removed liver lobes was measured through immersion method. The caudate lobe and right lateral lobe were remained. Then the peritoneum and the skin were closed. The control group received sham operation with no liver lobe removed. Both PH group and control group then underwent LFR assessment by PAT scanning (in the same method described in the previous normal rabbit part). Conventional liver function test measured the level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), total bilirubin (TB), direct bilirubin (DB), prothrombin time (PT) and activated partial thromboplastin time (APTT) in both PH group and control group.
2.5. Image reconstruction and analysis

All acquired data were first reconstructed in Labview (NI, USA) in real-time by using back-projection algorithm [33]. The reconstruction parameters can be adjusted in the program. The raw data were stored for offline processing by Matlab (R2016b, Mathworks, Inc., MA, USA). In the offline processing, 0.2-10 MHz filtering was implemented to remove high frequency noise and images were reconstructed in back-projection schemes [33].

Relative photoacoustic signal intensity (PSI<sub>re</sub>) were calculated to reflect the ICG concentration change as follows:

\[
PSI_{re}(t) = \frac{PSI(t) - PSI_{base}}{PSI_{base}}
\]

(1)

In order to quantify the kinetics of PSI<sub>re</sub> change after it reached the peak after ICG injection, an exponential decay model [34] was used as follows:

\[
PSI_{re}(t) = PSI_{re}(0)e^{-kt}
\]

(2)

A schematic diagram of this model is shown in Fig. 3 in red line. PSI<sub>re</sub>(t) is the relative PSI, which reflected the ICG concentration at time t, and PSI<sub>re</sub>(0) is the peak PSI<sub>re</sub> after ICG injection. The elimination time (t<sub>0</sub>) indicates the elapse time from PSI<sub>re</sub>(0) to the base line. Rate constant (k) gives the decay rate.

![Fig. 3. An exponential decay model was used to determine the PSI<sub>re</sub> change after it reached the peak after ICG injection (red line).](image)

2.6. Statistical analysis

Statistical analysis and graphical display of data were performed using GraphPad software (version 7.00; GraphPad Software, San Diego, CA, USA). All values were reported as mean ± standard deviation (SD) or median (range). Student t test was used to test the differences between control group and hepatectomy group for various parameters. The Pearson correlation coefficient were used to measure the correlation between temporal photoacoustic signal (Mean PSI trace) and PA or Sp phantom results as following:

\[
\rho(A, B) = \frac{1}{N-1} \sum_{i=1}^{N} \left( \frac{X_i - \mu_A}{\sigma_A} \right) \left( \frac{Y_i - \mu_B}{\sigma_B} \right)
\]

(3)

where \( \mu_A \) and \( \sigma_A \) are the mean and standard deviation of A, respectively, and \( \mu_B \) and \( \sigma_B \) are the mean and standard deviation of B.

A value of \( p < 0.05 \) was considered statistically significant.
3. Results

3.1. In vitro ICG measurement

ICG solution was encapsulated in a 3mm diameter transparent PVC pipe then embedded in the phantom (Fig. 4(a)). For the ICG encapsulated in the tissue-mimicking phantom, at ICG concentration of 0, 0.1, 0.2, 0.3, 0.5, 1.0mg/dl, the average PSI was 0.688 ± 0.010, 0.724 ± 0.023, 0.748 ± 0.036, 0.780 ± 0.036, 0.829 ± 0.037 and 0.958 ± 0.051, shown in green line in Fig. 4(b). with high goodness of linear fit ($R^2$=0.9984). In contrast, absorbance of the ICG-containing plasma from spectrophotometer (Sp) increased (0.120 ± 0.041, 0.171 ± 0.023, 0.277 ± 0.036, 0.393 ± 0.041, 0.566 ± 0.103, 0.952 ± 0.338) with ICG concentration change with high goodness of linear fit ($R^2$=0.9987) shown in blue line in Fig. 4(b). One set of PA images of different ICG concentration were shown in Fig. 4(c).

![Fig. 4.](image)

**Fig. 4.** Measurement of ICG in vitro phantom by spectrophotometer and photoacoustic tomography. (a). Tissue mimicking phantom setup. (b). Normalized absorbance of ICG by spectrophotometer (Sp, blue line) compared to the normalized PSI of ICG obtained by photoacoustic tomography (PA, green line). (c). Representative PA images showing a map of optical absorption from ICG with the indicated concentrations of ICG (0, 0.1, 0.2, 0.3, 0.5, 1.0mg/dl). a.u. = arbitrary unit, ICG = indocyanine green, Sp = spectrophotometer, PA = photoacoustic, PSI = photoacoustic signal intensity

3.2. In vivo ICG measurement in normal rabbits

A high intensity peak after ICG injection was observed and gradually decreased over time (Fig. 5.). High correlations were demonstrated between temporal PAS in vivo and PAS of blood sample in vitro ($r$=0.9707, $p$<0.001), and between temporal PAS in vivo and absorbance of blood sample in vitro ($r$=0.9517, $p$<0.001).
3.3. In vivo ICG measurement in partial hepatectomy rabbits

The control group showed a rapid decrease after the peak point, while the elimination of ICG for the PH group is slower (Fig. 6(a)). Clear difference in ICG clearance between the control and PH groups.
PH models was identified by PAT ($t_{01}$ vs. $t_{02}$, 199.8 ± 16.56s vs. 391 ± 23.23s, $p=0.0001$; $k_1$ vs. $k_2$, 0.0185 ± 0.0011 vs. 0.0064 ± 0.0023, $p=0.008$) (Figs. 6(b) & 6c). Difference in $PSI_{re}$ was also clear at $t_{01}$ (about 310s, $PSI_{re}=0.049 ± 0.068$ vs. 0.17 ± 0.071, $p=0.023$). No difference was shown at $t_{02}$ (about 500s, $PSI_{re}=0.048 ± 0.036$ vs. 0.055 ± 0.087, $p=0.863$) (Fig. 6(d)).

4. Discussion and conclusions

Liver function reserve (LFR) assessment plays a significant role for liver surgery and liver disease prognosis [1,2]. ICG clearance test is considered as the reference method for LFR assessment among various assessment techniques. However, spectrophotometry or PDD to perform ICG clearance test is either invasive or not very accurate [3,13]. PAT is a hybrid imaging technique that provides combination of functional information with real-time structural imaging. This study was carried out to investigate the feasibility of using PAT to perform ICG clearance test for LFR assessment. The in vitro and in vivo study results demonstrated the promising utility of this technique.

In our study, the light absorbance of ICG measured by spectrophotometer and its $PSI$ detected by PAT were first evaluated and compared in a tissue-mimicking phantom at wavelength of 805nm. And the tissue mimicking phantom study confirmed the linear relationship between absorbance of ICG and ICG concentration, consisted with previous study [35]. Taken spectrophotometer as the reference, $PSI$ of ICG detected by PAT also demonstrated a linear relationship with ICG concentration in the phantom study. Therefore, it provided the fundamental principle for using PAT to monitor ICG concentration change over time in vivo.

For in vivo study in normal New Zealand rabbits, we placed the PAT probe on the target peripheral vessel to monitor ICG concentration change over time for LFR assessment. B-mode ultrasound helped confirm this target vessel for measurement. Therefore, surrounding tissue influence to ICG measurement was excluded to a much greater extent compared to PDD [36]. Through peripheral vessel scanning, ICG clearance test for LFR assessment can be performed by PAT within the penetration depth limit of photoacoustic technique [37]. PAT examination was successfully performed for all the 7 normal rabbits and no side effects due to PAT examination were observed. Previous studies investigating ICG pharmacokinetics by spectrophotometric method or high-pressure liquid chromatographic assay revealed a simple monoexponential decline of ICG plasma concentration over time following low doses (0.5 mg/kg) to man [38] or laboratory animals [39–41], which supported our observations by PAT. On the other hand, in vivo real-time mean $PSI$ trace was in accordance with findings of the invasive gold standard spectrophotometry, which further confirmed the accuracy of PAT method for performing ICG clearance test for LFR assessment.

Based on the in vitro phantom and in vivo normal animal study results, we further built partial hepatectomy model that have direct clinical relevance to confirm the efficacy of this method for impaired LFR assessment. 70% hepatectomy has been reported to model extended hepatectomy with an insufficient remnant liver in various animals [42,43]. In our study, 70% of the whole liver volume was successfully removed in 4 rabbits in the PH group. The extended hepatectomy model had significantly increased ALT, AST and prolonged PT (Table 1), which verified the severely impaired liver function in these rabbits and suggested the successful establishment of the rabbit model of liver injury. Then ICG clearance between PH group and control group was monitored by PAT via the right central auricular artery in real-time. Compared to control group, the elimination time $t_0$ of ICG in PH group significantly prolonged and elimination rate constant $k$ obviously slowed down. It represented that 70% hepatocyte content loss consequently and significantly reduced the capacity of the liver to uptake ICG and excrete it into the bile. Similar results were found by Nathalie Brillant et al. in mouse model by MSOT [34]. Furthermore, we compared the mean and standard deviation values of $PSI_{re}$ for both groups at $t_{01}$ and $t_{02}$ time points. Clear difference between two groups was found at $t_{01}$, while no difference was seen at $t_{02}$.
This strongly suggests that PA imaging has the potential to become a clinical tool for quantitative prognosis of liver surgery and treatment.

| Table 1. Conventional liver function tests in both PH and Control group |
|---------------------------------------------------------------|
|                | ALT (U/L) | AST (U/L) | ALB (g/L) | PT (s) | APTT (s) | Direct Bilirubin (umol/L) | Total Bilirubin (umol/L) |
| PH (n=4)        | 165.9a    | 577.3a    | 33.1      | 10.6a  | 30.6     | 3.4                      | 5.1                     |
| Control (n=6)  | 42.5      | 17.4      | 39.1      | 8.2    | 23.7     | 2.0                      | 3.1                     |

Values are expressed as median (range), ap < 0.05, compared with Control group

Our study represents the first PA-based in vivo LFR assessment on central auricular artery. We also tested the minimal laser energy needed for detecting and monitoring the central ear artery in the rabbit model. The incident fluence at 805 nm was estimated to be 5 mJ/cm² to ensure a sufficiently high signal noise ratio (SNR) for PA imaging. However, we noticed that a considerably lower incident fluence (e.g., 0.5 mJ/cm²) also provided an acceptable imaging quality. Therefore, the use of much cheaper & safer solutions such as photodiode (PD) [29] or light-emitting diode (LED) [44] might be feasible, suggesting a future opportunity for PA imaging of LFR in humans.

Several limitations exist in our study. First, although the central auricular artery in our animal model was clearly visible by both the PA and ultrasound imaging, it would be necessary to build a hybrid photoacoustic/ultrasound imaging platform that allows for accurate image co-registration for human applications. Second, only 70% hepatectomy rabbit model was built in this study to verify the efficacy of the proposed PAT method for LFR assessment. Different degrees of hepatectomy and different chronic liver disease model should be considered for further validation. Third, a prospective clinical study in large population should be performed in the future.

In summary, using PAT to monitor ICG concentration change over time via peripheral vessel at wavelength of 805nm is a safe, accurate and effective method for LFR assessment, which is potentially promising for widely clinical application.

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**Disclosures**

The authors declare no conflicts of interest.

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