Quantitative Measurement of the Thyroid Uptake Function of Mouse by Cerenkov Luminescence Imaging

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Cerenkov luminescence imaging (CLI) has been an evolutional and alternative approach of nuclear imaging in basic research. This study aimed to measure the $^{131}$I thyroid uptake of mouse using CLI for assessment of thyroid function. Quantification of $^{131}$I thyroid uptake of mice in euthyroid, hypothyroid and hyperthyroid status was performed by CLI and $\gamma$-scintigraphy at 24 hours after injection of $^{131}$I. The $^{131}$I thyroid uptake was calculated using the equation: (thyroid counts − background counts)/ (counts of injected dose of $^{131}$I) × 100%. Serum T4 concentration was determined to evaluate the thyroid function. The radioactivity of $^{131}$I was linearly correlated with the CL signals in both in vitro and in vivo measurements. CLI showed a significant decrease and increase of $^{131}$I thyroid uptake in the mice in hypo- and hyperfunctioning status, respectively, and highly correlated with that measured by $\gamma$-scintigraphy. However, the percent thyroid uptake measured by CLI were one-fifth of those measured by $\gamma$-scintigraphy due to insufficient tissue penetration of CL. These results indicate that CLI, in addition to nuclear imaging, is able to image and evaluate the $^{131}$I thyroid uptake function in mice in preclinical and research settings.

Radioiodine has long been used for thyroid studies1, 2. Radioactive iodine uptake (RAIU) and thyroid scan are commonly used to evaluate patients with suspected thyroid disorders. RAIU that measures iodine metabolism in the thyroid gland is based on physiologic incorporation of radioiodide into the thyroid gland and is followed by determination of the fraction of the dose taken up in the gland over a given time period2. It has been widely used to assess the thyroid function in clinical practice. RAIU along with a thyroid scan is useful in differentiating the causes of hyperthyroidism, such as Graves’ disease, Plummer’s disease and subacute thyroiditis2. However, these nuclear medicine procedures are seldom applied on small animals in the preclinical studies due to the drawbacks of low spatial and temporal resolution of nuclear imaging and relatively high cost of instruments1, 2.

Cerenkov luminescence (CL) has been used recently in biomedical applications of imaging with clinically relevant medical isotopes3, 4. CL is a phenomenon which was first described by Pavel Alekseyevich Cherenkov in 19345. Charged particles traveling faster than the speed of light in a medium transfer their kinetic energy through interactions with the surrounding dipoles, in biological tissues mostly with water5. The randomly oriented water molecules will align with the passing super-relativistic charged particle and relax by releasing the transferred energy in the form of light5, 6. Cerenkov luminescence imaging (CLI) can be done using a sensitive camera optimized for low light condition which has a better resolution than any other nuclear imaging modality3. CLI has emerged quickly in preclinical molecular imaging with the use of various medically relevant radioisotopes, including $^{15}$O, $^{13}$N, $^{68}$Ga, $^{89}$Zr, $^{64}$Cu, $^{225}$Ac, $^{90}$Y, $^{131}$I, $^{124}$I, $^{18}$F and $^{74}$As, showing that the emitted radioactivity correlated well with the light output5–10.

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With the development of optical imaging techniques and the improvement of its equipped CCD systems, the sensitivity and accuracy for CL detection has made this method highly applicable and competent in preclinical basic research setting\(^1\). In addition, advantages such as lower cost than that of nuclear imaging modalities, without setup of radiochemistry facilities, nearly identical imaging procedures and short image acquisition time of radionuclide-based optical CLI have provided an alternative and potential approach for researchers who are not majored in the nuclear imaging but need to conduct the radionuclide-based research.

The preclinical use of CLI in the study of radioiodine uptake are becoming more common in recent years. Jeong et al. successfully showed the CLI and nuclear imaging of \(^{131}\)I and \(^{124}\)I uptake by mouse thyroid gland and by NIS-expressing thyroid cancer cells\(^1\). Using \(^{99m}\)Tc-pertechnetate, Boschi et al. demonstrated CLI of the thyroid glands and salivary glands of mice\(^1\). Spinelli et al. reported the first \(^{131}\)I CLI of a human thyroid gland with a therapeutic dose of \(^{131}\)I for the treatment of hyperthyroidism\(^4\). Hu et al. demonstrated the application of Cerenkov luminescence tomography for evaluating the \(^{131}\)I uptake function of mouse thyroid\(^5\). These results have shown the translational potential of CLI.

To the best of our knowledge, there is no report showing that CLI is able to quantitatively measure the thyroid uptake of radiiodide. In this study, we have approved that CLI is feasible to measure the \(^{131}\)I thyroid uptake in mice in different status of thyroid function.

**Materials and Methods**

**Mouse model of hypothyroidism and hyperthyroidism.** The animal manipulation and experiment procedures were reviewed and approved by the Institutional Animal Care and Use Committee of National Yang-Ming University. Six-week-old Balb/c male mice, purchased from National Laboratory Animal Center (NLAC, Taiwan), were housed in a temperature-controlled room with a 12-hour light-dark cycle and given access to food and water ad libitum. For induction of hypothyroidism, the mice were orally administered with methimazole (MMI, Sigma-Aldrich), 64 mg/kg in 200 \(\mu\)l distilled water, via gavage for 15 consecutive days. For induction of hyperthyroidism, the mice were intramuscularly injected with 2 \(\mu\)g of recombinant human thyroid stimulating hormone (rhTSH, Thyrogen, thyrotropin alfa, Genzyme) in 100 \(\mu\)l distilled water twice on two consecutive days. The control mice were administered with the same volumes of distilled water. Measurement of serum total T4 was carried out by using a Mouse/Rat Thyroxine ELISA kit (GenWay Biotech. Inc., San Diego, CA, USA) to assess the thyroid function.

**Correlation of CL signals and \(^{131}\)I activity in vitro and in vivo.** To assess the correlation of CLI signals and radioiodide activity in vitro, \(^{131}\)I was serially diluted into 500, 250, 125, 63, 32 and 16 \(\mu\)Ci (18.5, 9.3, 4.6, 2.3, 1.2 and 0.6 MBq) in 200 \(\mu\)l saline in Eppendorf tubes. CLI was carried out using an IVIS 50 imaging system (Perkin Elmer, Waltham, MA, USA) with a luminescence imaging setting of binning: 8, FOV: 12, f stop: 1, exposure time: 300 s. The signal of \(^{131}\)I-emitted CLI was analyzed using Living Imaging Software (Perkin Elmer) and shown in p/s/cm\(^2\)/sr.

To evaluate the correlation of injected \(^{131}\)I dose and CL signals in mouse thyroid, serial doses of \(^{131}\)I (0, 0.6, 1.2, 2.3, 4.6, 9.3 and 18.5 MBq in 200 \(\mu\)l saline) were intraperitoneally injected into mice followed by CLI 24 hours later. Before imaging, the mice were anesthetized by inhalation of 2% isoflorane mixed with oxygen. Quantification of CL signals from mouse thyroid was performed following the settings as described in in vitro study.

**\(^{131}\)I thyroid imaging and measurement of thyroid uptake function by \(^{\gamma}\)-scintigraphy and CLI.** \(^{131}\)I thyroid imaging and measurement of thyroid uptake function were carried out on six euthyroid mice, six mice in hypothyroid status and six in hyperthyroid status. The mice were intraperitoneally injected with 18.5 MBq of \(^{131}\)I, followed by \(^{\gamma}\)-scintigraphy and CLI 24 hours later. \(^{\gamma}\)-scintigraphy was performed by placing animals prone at a distance of 6 cm under a 4-mm pinhole collimator equipped on a gamma camera (Symbia E, Siemens). Image was acquired for 30 min. CLI was performed subsequently using an IVIS 50 imaging system with the same luminescence imaging settings mentioned above. The radioactivity of the thyroid gland was obtained from the region-of-interest (ROI) drawn along 85% isocount contour of the thyroid gland over the anterior neck. The background activity was measured over the head with a ROI of the same size as the thyroid gland. The photon flux of the CL of the thyroid gland and the background was obtained using the similar method as \(^{\gamma}\)-scintigraphy. The percent thyroid uptake of \(^{131}\)I was calculated using the equation \(\frac{\text{counts of injected }^{131}\text{I dose} - \text{background counts}}{\text{counts of injected }^{131}\text{I dose} 	imes 100\%}\). Correction of the decay of radiotracer was done for each measurement.

**Statistical analysis.** The numerical data were reported as means ± standard deviation (S.D.). Student’s t test was applied for comparison of serum T4 concentration and percent \(^{131}\)I thyroid uptake of each group with different thyroid function status. A significant difference was considered if the p value was less than 0.05.

**Ethical approval.** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not describe any studies with human participants performed by any of the authors.

**Results**

**Correlation of CLI signals and \(^{131}\)I activity in vitro and in vivo.** \(^{131}\)I with activity ranging from 500 to 16 \(\mu\)Ci (18.5 to 0.6 MBq) was aliquoted in the same volume (200 \(\mu\)l saline), placed in Eppendorf tubes, and then imaged by CLI using the IVIS 50 system. The luminescence intensity of \(^{131}\)I corresponded well with the doses of radiotracer (Fig. 1A). Within a ~5 cm\(^2\) ROI, 500 \(\mu\)Ci (18.5 MBq) and 16 \(\mu\)Ci (0.6 MBq) of \(^{131}\)I resulted in signal of approximately 3 \(\times\)10\(^5\) and 2 \(\times\)10\(^4\) p/s/cm\(^2\)/sr, respectively. A high correlation (R\(^2\) = 0.9994) was observed between the luminescence intensities and \(^{131}\)I activities (Fig. 1B).
To determine whether the accumulation of different doses of $^{131}$I in the thyroid gland of healthy mice can be accurately measured by CLI, doses of $^{131}$I from 0 to 500 $\mu$Ci (0 to 18.5 MBq) were intraperitoneally injected into the mice and CLI was performed 24 hours later. The results showed that the CL emitted from the thyroid gland was visually correlated with the amount of $^{131}$I activity injected (Fig. 1C). The signals from the thyroid ROI were ranging from $3 \times 10^4$ p/s/cm$^2$/sr (18.5 MBq $^{131}$I) to $4 \times 10^2$ p/s/cm$^2$/sr (0.6 MBq $^{131}$I). The CL signals were well correlated with the $^{131}$I doses ($R^2 = 0.9708$) (Fig. 1D). The results suggested that the minimum dosage of $^{131}$I CLI imaging of mouse thyroid gland is 125 $\mu$Ci (4.6 MBq).

In vivo $^{131}$I CLI and $\gamma$-scintigraphy of the mice in different thyroid function status. Mouse models of hypothyroidism and hyperthyroidism were established by treatment with MMI and Thyrogen, respectively. Total serum T4 was measured to confirm the successful induction of hypothyroidism and hyperthyroidism. The results showed that the serum T4 level was significantly higher in rhTSH-treated mice (~14.8 ± 3.5 $\mu$g/dl) and significantly decreased in MMI-treated mice (~2.1 ± 0.4 $\mu$g/dl), as compared to that in the euthyroid subjects (~3.7 ± 0.9 $\mu$g/dl) ($p < 0.05$) (Fig. 2).

The mice in hypothyroid or hyperthyroid status were intraperitoneally administrated with 500 $\mu$Ci (18.5 MBq) of $^{131}$I, followed by CLI and $\gamma$-scintigraphy 24 hours later. The CL images showed obvious reduction or increase of luminescence signals in the thyroid glands in hypothyroid or hyperthyroid status, respectively, as compared to the euthyroid mice (Fig. 3). ROI analysis showed that the CL signals were $2 \times 10^5$ ~ $2 \times 10^6$ p/s/cm$^2$/sr for the...
hypothyroid mice, 1.2 ~ 1.4 × 10^6 p/s/cm^2/sr for the hyperthyroid mice and 4 ~ 7 × 10^5 p/s/cm^2/sr for the control euthyroid mice. A similar result was observed by γ-scintigraphy, showing reduced or increased γ signal in the thyroid glands of mice in hypothyroid or hyperthyroid status, respectively (Fig. 3). The radioactivity (counts per second, cps) obtained from the ROIs of the thyroid glands were 10 ~ 50 cps for hypothyroidism, 200 ~ 300 cps for hyperthyroidism and 70 ~ 130 cps for euthyroid mice. The percent 131I uptake of the thyroid measured by CLI were 0.6 ± 0.3%, 3.0 ± 0.6% and 7.3 ± 0.6% for hypothyroid, euthyroid and hyperthyroid status, respectively. By γ-scintigraphy, the percent 131I uptakes of thyroid were 2.7 ± 1.7%, 12.9 ± 3.3% and 31.9 ± 5.3% for hypothyroid, euthyroid and hyperthyroid status, respectively (Fig. 4A and B). The value of the percent thyroid uptake for each group measured by CLI is about one-fifth of that measured by γ-scintigraphy. The percent thyroid uptake of 131I measured from the two imaging modalities were well correlated and had a high linear regression (R^2 = 0.9621) (Fig. 4C), indicating the feasibility and reliability of CLI for the measurement of 131I thyroid uptake.
Discussion

In this study, we have demonstrated the feasibility of using CLI for quantification of $^{131}$I thyroid uptake function in mice. The results reveal a high linear correlation between $^{131}$I activity and the CL signals in the thyroid gland of the mice. In hypothyroid or hyperthyroid mice, CLI provides a reliable quantitative measurement of $^{131}$I thyroid uptake, which is comparable with the uptake measured by $\gamma$-scintigraphy and compatible with the thyroid function status of the mice.

The hypothyroid and hyperthyroid mice were induced by administration of MMI and rhTSH, respectively. Administration of rhTSH into normal subjects resulted in a significant increase in serum T4 at 8 h, reached to peak at 24 h and remained the high T4 level for at least 4 days\(^{16}\). Increased radioiodine uptake by normal subjects has also been observed at 6 and 24 hours after rhTSH treatment\(^{17}\). Serum T3 and T4 concentrations in euthyroid mice are strikingly increased at 6 hour after rhTSH treatment\(^{18}\). In the current study, the total T4 in euthyroid mice was $3.7 \pm 0.9 \mu$g/dl (n = 6), comparable with a range of 4 ~ 8 $\mu$g/dl for balb/c mice in earlier literatures\(^{19, 20}\) and with the data outlined by the Mouse Phenome Database at the Jackson laboratory. At 24 hours after two injections of rhTSH (totally 4 $\mu$g), serum T4 was effectively elevated by four folds ($14.8 \pm 4.2 \mu$g/dl). The effect of rhTSH on the radioiodine uptake of euthyroid mice has also been studied with $^{125}$I, which showed decreased uptake during the first 3 to 5 hours after treatment and then increased at 13 hours\(^{45}\). Based on these reports, 24 hours after rhTSH treatment was assumed to be the most suitable time point for the $^{131}$I imaging in mice. Notably, the mice with rhTSH stimulation showed a two-fold increase in $^{131}$I accumulation in thyroid glands as compared to that in control mice in this study. MMI has been used to treat hyperthyroidism caused by Graves’ disease for more than half a century, and is able to effectively induce hypothyroidism in euthyroid rodents\(^{21, 22}\). The mice were treated with ~1.6 mg (64 mg/kg) of MMI for 15 consecutive days. The serum T4 concentration (2.1 $\pm$ 0.4 $\mu$g/dl) was reduced to almost half of that in control mice (3.7 $\pm$ 0.9 $\mu$g/dl). $^{131}$I uptake by the thyroid gland measured by both CLI and $\gamma$-scintigraphy showed a decrease by one fifth of that of the euthyroid mice. In summary, hypothyroidism or hyperthyroidism of mice induced by MMI or rhTSH, respectively, is able to be monitored by $^{131}$I CLI with the same efficacy as $\gamma$-scintigraphy.

Clinical PET examinations and the signals obtained by the endoscopic CLI to detect gastrointestinal malignant neoplasms\(^{31}\). These newly developed technology of optical imaging instruments are expected to improve both the quality of $^{131}$I imaging of thyroid gland and the measurement of $^{131}$I thyroid uptake by CLI as well.

In conclusion, we have demonstrated the feasibility of using CLI more effectively in clinical experiments by improving the image equipment. Spinelli et al. optimized the CCD detector for the near infrared (NIR) range, which contained a smaller number of Cerenkov photons, but had better tissue penetration ability. The sensitivity was improved up to 35%, and this advantage was more significant when the Cerenkov source is being imaged at a deeper location within the animal\(^{37}\). CCD systems with enhanced sensitivity for detecting low-light levels, such as intensified CCD (ICCD) and electron-multiplying CCD (EMCCD), have been used in recent studies\(^{14, 28, 29}\). These highly sensitive intensified CCD systems have recently been coupled to optical fiber allowing the demonstration of preclinical endoscopic surgery\(^{36}\). The results of a previous clinical study showed a good consistency with clinical PET examinations and the signals obtained by the endoscopic CLI to detect gastrointestinal malignant neoplasms\(^{31}\). These newly developed technology of optical imaging instruments are expected to improve both the quality of $^{131}$I imaging of thyroid gland and the measurement of $^{131}$I thyroid uptake by CLI as well.

In this study, we have demonstrated the feasibility of using CLI for quantitatively assessment of thyroid uptake function of radioiodide. CLI is able to quantify the $^{131}$I accumulation in the thyroid glands of mice with different functional status. The thyroid uptake of $^{131}$I measured by CLI is comparable to that obtained by $\gamma$-scintigraphy. In addition to nuclear medicine imaging modalities, CLI is useful to assess the thyroid uptake function of radioiodine in preclinical and basic research settings.

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**Author Contributions**

C.C.K., W.S.H. and R.S.L. conceived and designed the experiment. Z.M.H., C.C.K., Y.J.H., C.W.H., J.J.L., Y.A.C. and C.W.C. performed the experimental work. B.H.Y., W.S.H., L.H. and C.C.K. analyzed the data. C.C.K. and Y.J.H. wrote the manuscript.

**Additional Information**

**Competing Interests:** The authors declare that they have no competing interests.

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