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Optical imaging of Cerenkov light generation from positron-emitting radiotracers

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Abstract

Radiotracers labeled with high-energy positron-emitters, such as those commonly used for positron emission tomography (PET) studies, emit visible light immediately following decay in a medium. This phenomenon, not previously described for these imaging tracers, is consistent with Cerenkov radiation and has several potential applications, especially for *in vivo* molecular imaging studies. Herein we detail a new molecular imaging tool, Cerenkov Luminescence Imaging, the experiments conducted that support our interpretation of the source of the signal, and proof-of-concept *in vivo* studies that set the foundation for future application of this new method.

1. Introduction

Positron emission tomography (PET) has emerged as a cornerstone imaging modality in the medical community. PET is a sensitive, noninvasive functional and molecular imaging tool that maps the distribution of picomolar levels of tracers labeled with radionuclides such as ¹⁸F, ¹⁵O, ¹³N, and ¹¹C. The most widely used tracer is the ¹⁸F-labeled analog of glucose, 2'-deoxy-2'-[¹⁸F]fluoro-D-glucose (FDG). Metabolically active cells—such as those in the brain, heart, or a malignant tumor—are glucose avid and, thereby, accumulate FDG at a higher rate than other tissues (Warburg 1956). As a marker of cellular glucose consumption and metabolic rate, FDG is used to assess cancer patients, to locate metastatic lesions with higher sensitivity than anatomical imaging modes, and monitor therapeutic response (Kelloff 2005, Mikhael 2005, Shankar 2006). Emerging tracers promise to provide additional information regarding tumor status and response to treatment; these include ones targeting proliferation (e.g., fluorothymidine, FLT), hypoxia (e.g., fluoromisonidazole, FMISO, or fluoroazomycin arabinoside, FAZA), and perfusion (e.g., ¹⁵O-water or ¹⁸F-galacto-RGD).

In the dominant mode of ¹⁸F decay, the nucleus emits a high-energy positron with energy of up to 635 keV (average energy of 250 keV). Due to interactions with tissue in PET applications, the positron scatters, loses energy, and eventually annihilates with an electron that may be a millimeter or more away from the decay event. The annihilation produces a pair of 511 keV photons that ultimately reach the detector ring of the PET scanner, which thus maps the position of the annihilation. The initial velocity of the charged, high-energy positrons often momentarily exceeds the speed of light in tissue and therefore satisfies the requirements for the production of Cerenkov radiation.

Cerenkov radiation is produced when a charged particle travels with a velocity that exceeds the speed of light through an insulating medium (Jelley 1955, Elrick 1968, Ross 1969, L'Annunciata 2003). The charged particle induces a local polarization along its path through the medium, and radiation is emitted during the return to equilibrium. At a particular angle that allows for constructive interference, the emitted light forms a coherent wavefront and can propagate to be seen at a distance. Cerenkov radiation is produced in a continuous spectrum from the near ultraviolet through the visible spectrum, distributed inversely proportional to the square of the wavelength. Early work in Cerenkov imaging of β -emitting radionuclides noted potential applications, but also the limits due to the low photon output which complicated detection (Elrick 1968). Herein, we demonstrate that positron-emitting (β^+) radionuclides can produce visible light consistent with Cerenkov radiation, with a continuous spectrum that is weighted towards the ultraviolet and blue bands of the electromagnetic spectrum. Further, we introduce Cerenkov Luminescence Imaging (CLI), a novel imaging method that is suitable for *in vivo* imaging in mice.

2. Methods and Results

All optical imaging experiments were conducted using Xenogen devices (IVIS 100 or 200, Caliper Life Sciences, Alameda, CA). Further, all animal experimentation was conducted under approved IACUC research protocols of Millennium Pharmaceuticals, Inc.

Preliminary demonstration of the detection of Cerenkov luminescence was performed by scanning plates with various activities of positron-emitting isotopes. Wells of ^{18}F (maximum β^+ energy 0.635 MeV, average β^+ energy 0.25 MeV) and ^{13}N (maximum β^+ energy 1.19 MeV, average β^+ energy 0.49 MeV) were prepared with activities of 1, 10, or 100 μCi and scanned with an integration time of 10 seconds using the bioluminescence “channel”—that is, no fluorescence filters or excitation light were applied. ^{18}F and ^{13}N images are shown in Figures 1A and 1B, respectively. For both isotopes, the measured CCD signal (luminescence or radiance), after background subtraction (using the signal from an area of an adjacent empty well), increased proportional to the activity ($R^2 = 0.99$), which is quantified in Figure 1C. In addition, the higher energy positron-emitter, ^{13}N , produced more observable light than ^{18}F . At 100 μCi , ^{13}N produces approximately $6.5\times$ more light than ^{18}F .

We performed a subsequent scan with opaque paper over the sample, and no signal was detected. High-energy photons would pass through paper; therefore, the loss of signal confirms that the signal detected on the CCD was visible light. As a second method to prove that the signal is not the result of direct gamma detection, a vial of 1 mCi technetium-99m ($^{99\text{m}}\text{Tc}$), a gamma emitter with minimal beta emission, was scanned, and no light was detected. Finally, to demonstrate that an active positron-emitter is required, a vial of 200 μCi ^{18}F -FDG was scanned after 13 half-lives, and no signal was detected. Thus the luminescence signal is in the visible spectrum and related to the activity of the β^+ source.

The speed at which light propagates through a medium is proportional to the refractive index, η . To test the dependence of observed light on η , we prepared a series of solutions with 0, 10, 20, and 40% glucose (by mass) with estimated η of 1.33, 1.35, 1.36, and 1.40, respectively (Tolman 1906). Each solution was doped with 209 μCi of FDG. As demonstrated in Figure 2A, the light output increases as refractive index increases, consistent with the reduction in the speed of the propagation of light in the medium and an amplification of the Cerenkov radiation.

To estimate the spectral characteristics of light emitted during ^{18}F decay, a series of four filtered images of a sample of ^{18}F in water were acquired and quantified and the light output from each is plotted in Figure 2B. The filter set used is the standard set installed on the IVIS system: 515–575 nm (GFP), 575–650 nm (DsRED), 695–770 nm (Cy5.5), and 810–875 nm (ICG). A scan

was also taken with no filter, and by subtracting the data from the other four spectral bands, used to estimate the light intensity in the blue part of the spectrum. Correction for the wavelength-dependent quantum efficiency of the CCD camera has been applied. As expected for Cerenkov radiation, the majority of the light is produced in the blue portion of the spectrum and follows an inverse relationship with the square of the wavelength. Following, an experiment was conducted to monitor the decay of luminescence for a series of FDG solutions with activities of 14, 28, 55, 110, and 220 μCi . For a period of 140 minutes, 28 luminescence images were acquired with an integration time of five minutes each. The background-corrected signal intensity data was fitted to a monoexponential, and the luminescence half-life, $t_{1/2}$, was determined by

$$t_{1/2} = \frac{\ln(2)}{\tau}, \quad [1]$$

where τ was the decay constant and equaled 0.0067 min^{-1} ($R^2=0.99$). The $t_{1/2}$ of the luminescence signal was 103.5 min, which is consistent with the expected value for ^{18}F decay (109.8 min).

Figure 3 shows the light detected from a mouse bearing a CWR22-RV1 (human prostate tumor) xenograft. The animal was injected with 270 μCi FDG, allowed a 1 hr uptake period, anesthetized by 2% isoflurane delivered in medical air, and scanned in the optical imaging system using an integration time of 1 minute. Light was detected in the tumor, where a high level of FDG is accumulated, as well as near the snout, eyes, and brain. The photon flux in a region-of-interest (ROI) containing the tumor was 4.7×10^5 photons/s. ROIs above the tumor (on the animal) and in the image background (away from the animal) recorded values of 0.42×10^5 and 0.11×10^5 photons/s respectively.

Figure 4A shows an FDG PET scan on two mice, each bearing a flank colon tumor. These tumors were generated from primary human tissues excised during surgical resection and have demonstrated adequate growth and FDG uptake kinetics for *in vivo* drug discovery applications. The 10-minute PET scan was acquired on a dedicated small animal scanner (microPET® R4, Siemens Medical) following a 6-hour fast and a 1-hour uptake period (300 μCi FDG i.v. in 100 μL PBS). The data is shown as the maximum intensity projection (MIP) from the sagittal orientation. Immediately preceding the PET scan, the animals were imaged in the optical scanner using a 1 minute integration time. The optical image is shown in Figure 4B; note the light detected through the entire animal as well as strongly in the region of the bladder and tumor.

On the PET image, it was observed that one tumor had higher signal than the other, and that is reflected in the quantification. Standard PET measurements were made including standardized uptake value (SUV) (Zasadny 1993) and percent injected dose per gram (%ID/g) of the injected FDG dose in the tumor. The SUV was 2.4 and 1.9, and the %ID/g was 9.8% and 7.4% (left and right, respectively). From the optical image, a similar intensity difference was measured: 4.4×10^5 photons/s versus 2.3×10^5 photons/s, left and right, respectively.

3. Discussion

The demonstration of visible light emission during the radioactive decay of positron-emitting radionuclides represents a new method of molecular imaging, which we have termed Cerenkov Luminescence Imaging (CLI). Figure 3 and Figure 4 demonstrate clearly the potential for *in vivo* CLI with widely available optical instrumentation as a molecular imaging modality.

Figures 1A, 1B, and 1C demonstrate that (1) increased activity increases luminescence by increasing the number of events and (2) that higher energy positron-emitters produce more luminescence. We also confirmed that other ^{18}F tracers— $[^{18}\text{F}]\text{NaF}$ and 3'-deoxy-3'- $[^{18}\text{F}]$ fluorothymidine (FLT)—emit light, showing that the observation is linked with the positron-emitter rather than the chemical nature of the tracer. Further, the role of the positron-emitter is supported by the observed luminescence signal decay as a function of time.

Another explanation considered for the source of the signal was bremsstrahlung, which is radiation emitted during the deceleration of high-energy, charged particles due to interactions with nuclei in the surrounding medium. There is a low probability of significant bremsstrahlung radiation production in low atomic number (Z) materials (such as water and tissue), and the bremsstrahlung would not change as a function of the refractive index of the material as we have observed in Figure 2A. Furthermore, the spectral distribution of the observed signal is inconsistent with bremsstrahlung radiation. That said, there are examples of bremsstrahlung imaging used with high energy beta emitters such as ^{32}P and ^{90}Y and detection resulting high energy photons. A major distinction is that bremsstrahlung imaging is not performed using CCD detection but rather using gamma detection equipment, such as in single photon emission computed tomography (SPECT) imaging (Clarke 1992). It is worth noting that these high-energy β^- emitting radionuclides should also generate Cerenkov radiation, which would be detectable using the methods demonstrated herein; additional studies are ongoing to characterize the relative role of each process.

The high energy ^{18}F or ^{13}N positrons are governed by relativistic physics and eject from the nucleus near the speed of light, c . The speed of light in a fluid or tissue medium, v_m , is less than in a vacuum (i.e., $v_m < c$) and for some fraction of the positron energy spectrum, the positron velocity is initially greater than the speed of light in the medium. The reduction in the speed of light in a medium is due to the interaction of light with the material and is proportional to the refractive index, η , of the medium:

$$v_m = \frac{c}{\eta}. \quad [2]$$

Water has an η of 1.33, thus the speed of light in water is $v_m \approx 0.75 c$. Biological tissue has an η of ~ 1.36 – 1.45 (Mobley 2003), so the speed of light in tissue is $v_m \approx 0.7 c$. When the speed of a particle nears the speed of light, the relativistic kinetic energy, E , is calculated by:

$$E = mc^2 \left[\frac{1}{\sqrt{1 - v^2/c^2}} - 1 \right]. \quad [3]$$

Here m is the particle mass and v is the velocity. Solutions to Equation 3 reveal that a proportion of the positrons are traveling faster than the speed of light in the medium. For example, for the 0.635 MeV maximum energy of the ^{18}F positron, the velocity, v , is $0.895 c$. For a 1.19 MeV positron from ^{13}N , the velocity is $0.954 c$. During the short time that the charged positron is traveling faster than the speed of light in tissue, there is a burst of Cerenkov radiation. The threshold energy for production of Cerenkov radiation by a positron in tissue ($v = v_m$ in Eq. 3) is 0.26 MeV (Jelley 1955, Elrick 1968, Ross 1968). This is approximately equal to the mean energy of the distribution for ^{18}F positrons. During transit, the positron will lose energy, eventually falling below the threshold, and will no longer satisfy the Cerenkov criteria. The amount of light (i.e., number of photons) produced by a charged particle with energy exceeding

the Cerenkov threshold was first calculated by Frank and Tamm (1937), who shared the Nobel Prize with Cerenkov in 1958. Later, Ross (1969) noted analytical solutions are complicated by range and energy degradation factors but published a table of some numerically integrated solutions to the Frank-Tamm equations. Interpolating from the table, we estimated that a 0.635 MeV positron would produce approximately 20 photons in the 250–600 nm wavelength range. This is likely a maximum estimate as Collins and Reiling (1939) measured 40 photons from a 1.9 MeV electron whereas an estimate based on the Ross table is 89. Estimates of Cerenkov light emitted from the ^{18}F used in these studies are approximate and require additional modeling; however, an initial estimate based on the Ross (1969) tables and the energy spectrum of the decay is 3 photons per decay (or photons per second per Bq) of ^{18}F activity in water. Experiments and more detailed calculations are being performed to confirm this estimate and to model the effects of photon absorption in the medium and the efficiencies of the CCD camera.

For optical imaging studies, it is vital to consider the role of tissue absorption and scattering if *in vivo* imaging applications are to be feasible. As expected for Cerenkov radiation, the majority of the light is produced in the blue portion of the spectrum and follows an inverse relationship with the square of the wavelength. An important consideration is that Cerenkov light extends into the green and red region of the EM spectrum as shown in Figure 2B. Whereas blue light has a short range in tissue, limiting *in vivo* applications, green and red light have deeper tissue penetration, increasing the range of applications for imaging studies. But, like other optical imaging methods, the absorption and scattering of the light within the tissue results in relatively low levels of light output and a blurring effect. We have considered that future study is warranted to determine if for deep structures, such as tissues *in vivo*, the Cerenkov radiation may stimulate local autofluorescence that is then subsequently detected. While this phenomenon may increase depth of light penetration, it may also complicate the localization of signal. In addition, we are performing experiments to demonstrate that the Cerenkov radiation can be used with UV-excitable fluorophores, such as lanthanides, and the Stokes shift results in an enhanced release of light in a tissue-penetrating band.

The first demonstration of CLI as a viable *in vivo* imaging method is presented in Figure 3. Although the FDG broadly distributes through the animal, the image is thresholded to show the enhanced signal—hence accumulation of FDG—in the xenograft, a CWR22-RV1 (human prostate tumor) xenograft injected subcutaneously. We have previously shown the use of the CWR22-RV1 model for *in vivo* FDG PET imaging in conjunction with bortezomib treatment (Zhang 2005). The CWR22-RV1 xenograft is passaged by tumor fragment from animals bearing tumors and the cells have not been engineered for bioluminescence by luciferase, thus only the FDG and Cerenkov luminescence could be responsible for the emitted light. Although the optical image shown in Figure 3 is compelling evidence of luminescence imaging with Cerenkov radiation, it is interesting to compare this method with PET scanning, since they are both imaging the same tracer. Of course, the method and process of detection and image generation for PET and CLI are different. The light produced as a result of Cerenkov radiation would precede the annihilation event and production of paired photons. Further, minimal tissue absorption and scattering will occur on the 511 keV photons, whereas this is likely to be a major factor in the CLI method. Despite these caveats, both imaging methods rely on the radiotracer in tissue, and one would expect significant agreement between data acquired from each scan.

Note the intensely hot bladders in the PET image and the high degree of luminescence in this region on the optical image. This data supports that the optical measurement, at least for relatively superficial tissues, is a good surrogate for the radiotracer accumulation in the tissue. Experiments are currently being conducted to compare the relative level of sensitivity and performance of PET and optical imaging with PET tracers in drug treatment studies. Modern preclinical PET scanners offer up to ~10% sensitivity, which we expect will greatly exceed

the sensitivity of an optical CLI experiment for *in vivo* applications due to tissue absorption and scattering. Thus as a direct comparison to PET scanning, CLI may best be used as a high throughput proof-of-concept method. For example, in a typical CLI scan, up to 5 animals can be imaged simultaneously in a 3–5 minutes; conversely, PET scans are often 1–2 animals in 10–20 minutes. Conservatively, CLI is 4× faster—but could be 30× depending on experimental design—than small animal PET scanning.

4. Conclusions

We anticipate that this finding could have a substantial impact in the areas of optical and radio-imaging and molecular imaging (Pierce 2008, Weissleder 2008), especially for researchers who are combining the modalities, and may provide a cost-effective solution for laboratories currently limited to optical imaging techniques. All positron-emitting radionuclides used in biomedical imaging have sufficient energy to result in Cerenkov radiation that can be observed with sensitive optical imaging equipment. In fact, ^{18}F is one of the lowest energy positron-emitters, and the use of higher energy positron emitting radionuclides could improve detection and sensitivity. These radioisotopes include ^{15}O , ^{68}Ga , and ^{124}I . Other radionuclides, including some of those used for SPECT imaging and for radioimmunotherapy (e.g., ^{131}I , ^{90}Sr , ^{89}Y , or ^{67}Cu), should also produce Cerenkov signals, increasing the range of applications for CLI. Optical imaging applications could be optimized to allow for the *in vivo* monitoring of radioimmunotherapy rapidly and at much lower cost to existing systems. The sensitivity limitations due to scattering and absorption would limit the clinical use of CLI for whole body tomography; however, for preclinical studies it is conceivable that CLI experiments may provide a high-throughput precursor / validation for *in vivo* PET studies.

In addition, we are considering further applications for this phenomenon. For one, it is worth noting that the Cerenkov radiation is produced during the initial decay process, thereby more localized to the decay event than the annihilation event tracked by PET scanning. We are investigating the use of CLI for high-resolution tissue imaging analogous to autoradiography however using the Cerenkov luminescence for detection. For a final example, there are strategies that can be explored to utilize PET probes as imaging agents as well as energy sources to excite fluorophores or other photo-excitabile/cleavable molecules. Current work has focused on the use of lanthanide probes, which are excited in the ultraviolet range but emit in the tissue-penetrating 615 nm range. In addition, we are pursuing the concept of Cerenkov luminescence tomography (CLT), in which the propagation of optical photons inside tissues generated by the Cerenkov mechanism is modeled and the location of sites of optical photon emission (corresponding to the location of β -emitting radiopharmaceuticals) are traced back inversely from the surface measurements with an iterative reconstruction method. CLT could be used to image the distribution of β^+ or β^- -emitting radiotracers or to validate complex reconstruction algorithms for 3D bioluminescence tomography. More than a low-cost PET alternative, detection and utilization of the Cerenkov radiation is a new approach to *in vivo* molecular imaging experiments.

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Figure 1. The luminescence detected from well plates of 1, 10 and 100 μCi of ^{18}F (A) or ^{13}N (B) following a 10 second image acquisition. The quantification of the radiance, plotted in C, revealed that the higher energy positron-emitter produced significantly more light. For example, at 100 μCi , ^{13}N produces approximately 6.5 $\times$ more light than ^{18}F .

Figure 2A

Figure 2B

Figure 2.

A: The light output from solutions of solutions with refractive indices of approximately 1.33, 1.35, 1.37, and 1.41 (0, 10, 20, and 40% glucose) spiked with 209 μ Ci FDG. The light output increase as a function of glucose concentration (increase in refractive index) is consistent with the reduction in the speed of the propagation of light in the medium and an amplification of the Cerenkov radiation.

B: Results from a series of filtered scans on the wavelength of light detected from positron-emitter samples. The majority of the light is produced in the blue portion of the spectrum, which is expected for Cerenkov radiation, and follows an inverse relationship with the square of the

wavelength. Cerenkov light extends into the green and red region of the EM spectrum, which could increase the range of applications for *in vivo* imaging studies.

Figure 3.

Optical scan of a mouse bearing a CWR22-RV1 xenograft following injection of 270 μCi FDG. Luminescence was detected throughout the animal, which is consistent with the broad distribution of FDG; however, for display, the image is thresholded to highlight the tumor region. In the tumor, the measured luminescence is 11 $\times$ the signal measured in a region above the tumor and 47 $\times$ the signal from the image background.

Figure 4A and 4B (bottom)

4A

4B

Figure 4.

A sagittal maximum intensity projection (MIP) of an FDG PET scan on two mice bearing flank colon tumors derived from primary human tissue (A). The image were injected with 300 μCi FDG and prior to the PET scan (10 min) were imaged in the optical scanner (1 min exposure). The optical scan (Figure 4B) showed different degrees of light output that were consistent with the quantification from the PET scan. The SUV was 2.4 and 1.9, and the %ID/g was 9.8% and 7.4% (left and right, respectively). From the optical image, a similar intensity difference was measured: 4.4×10^5 photons/s versus 2.3×10^5 photons/s, left and right, respectively. In the optical scan, the image was thresholded to show the light emanating from the animal, demonstrating the full distribution of the FDG tracer as shown in PET scans.