The Molecular Imaging Center (MIC) is the youngest research center at NIRS, established in 2006 for research and development on clinical applications of radiation, especially in the field of nuclear medicine, including positron emission tomography (PET), single photon emission tomography (SPECT) and internal radiation therapy. Research on these is based on collaboration among diverse areas as follows:

1. Drug design for target-selective delivery (molecular probe), labeling of the molecular probe with suitable radionuclide for diagnosis/therapy, and radionuclide production.
2. Development of three-dimensional gamma-ray measurement systems such as PET and SPECT, including hardware and software.
3. Clinical application for diagnosis/therapy of tumors, psychiatric and neurodegenerative disorders, and so on.

The MIC has four research groups: the Molecular Probe Program, the Biophysics Program, the Diagnostic Imaging Program, and the Molecular Neuroimaging Program.

The MIC also promotes research on magnetic resonance imaging (MRI), X-ray-CT imaging, and optical imaging; these radiological imaging techniques are an integral part of diagnostic imaging in nuclear medicine. The development of PET-CT has realized fusion images of anatomy and functionality that have resulted in a synergic progress in diagnostic imaging. Recently, PET-MR has become commercially available, and it will surely bring unexpected advances in the clinical field.

**Molecular Probe Program**

1) Radiolabeling technique

We developed a method for preparing 2-[\(^{11}\)C]methoxypyridine using [\(^{14}\)C]methyl triflate as a radiolabeling agent. Using this method, we synthesized a novel PET probe with a reliable and high radiochemical yield and it is expected to be put to routine clinical use. We carried out a joint research project with a pharmaceutical company to develop PET probes for brain neurotransmitters and found a promising candidate which was synthesized using [\(^{14}\)C]cyanide as a labeling agent.

A convenient route was determined for producing [\(^{11}\)C]formaldehyde with a reproducible radiochemical yield and sufficient amount of radioactivity for labeling. Using [\(^{11}\)C]formaldehyde, we developed a novel technique for radiosynthesis of [\(^{11}\)C]oligopeptide. On the other hand, a labeling method directly using cyclotron-produced [\(^{18}\)F]KF aqueous solution was established for synthesizing new [\(^{18}\)F]peptide PET probes for tumor imaging.

**Fig. 1** PET study of [\(^{11}\)C]ITMM for human brain
2) Development of novel PET probes

We designed and labeled novel analogs of hippuric acid for PET imaging of organic anion transporters. After labeling the analogs by $^11$Cacylation or $^11$Cmethylation, the radioactive products were successfully obtained. Preliminary evaluation showed that one candidate product had promising properties as an in vivo PET probe and was worth further evaluation. This probe had potentials for measuring the functional activity of organic anion transporters in brain and multi-drug resistance protein 4 in heart. In addition, we demonstrated that an $^11$Camino acid agent $^11$CAIB is useful for imaging the function of the blood-brain-barrier in inflammatory and glioma animal models.

Using PET with $^{18}$FEDAC, a radioligand for translocator protein (18 KDa), we successfully visualized lung inflammation and non-alcoholic fatty liver disease. On the other hand, dozens of PET candidates for metabotropic glutamate 1 receptor were designed and screened. Three promising probes showing high specific binding in brain were selected for an in vivo imaging study in primate brain and its periphery. Of these PET probes, $^11$CITMM is undergoing clinical study for the imaging and quantitative analysis of metabotropic glutamate 1 receptor in human brain (Fig.1).

3) Production of SPECT, non-standard PET and alpha-emitting radionuclides

A basic study for the production method of $^{99m}$Tc, an important radionuclide in nuclear medicine, by proton-induced nuclear reactions was carried out. Several $^{99m}$Tc-labeled radiopharmaceuticals were synthesized and analyzed successfully.

A system producing $^{89}$Zr was developed and the efficient production procedures including extraction from the cyclotron target and purification were determined. Sufficient radioactivity of $^{89}$Zr was produced for radiolabeling and an evaluation study on animals was made. In addition to the positron-emitting isotopes, we furthermore developed a remotely controlled system for producing $^{211}$At as a useful short-lived alpha-emitting isotope for medical applications.

4) Production of useful PET probes for clinical application

For the past two years, we have improved and optimized the procedures of operation and quality control, and achieved standardization of many PET probes which are routinely produced in NIRS for human use. In addition, we prepared many kinds of documents for validation of the regular production and quality control methods for safe administration into human subjects, and for evaluation of the toxicity and radiation dosimetry of PET probe for clinical applications. Three new PET probes were approved by the IRB for clinical research in last fiscal year.

5) Contribution to quality control of clinical PET within Japan

We performed quantitative analysis and provides certificates for chemical impurities in $^{18}$FFDG and other radiopharmaceutical preparations which were produced in other PET facilities in Japan.

Biophysics Program

The Biophysics Program aims at development of next generation PET technologies and of methods for quantitative analyses of in vivo imaging. PET plays important roles in clinical diagnosis and molecular imaging research, but there are several potential points for which big improvements could be made including resolution, sensitivity and cost. Quantitative analyses of PET data are also important to measure physiological functions.

1) Next generation PET technologies

The Imaging Physics Team carries out basic studies on instrumentation, image reconstruction (Fig.2) and data corrections to improve image quality and quantity in nuclear medicine. A depth-of-interaction (DOI) detector will be a key device to get any significant improvement in sensitivity while maintaining high spatial...
resolution. DOI measurement also has a potential to expand application of PET to new fields because it allows for more flexible detector arrangement. As an example, we are developing the world’s first, open-type PET geometry OpenPET, which is expected to lead to PET imaging during treatment. We have developed a small prototype to show a proof-of-concept of OpenPET imaging. The DOI detector itself continues to evolve with the help of recently developed semiconductor photodetectors, often referred to as silicon photomultipliers (SiPMs). We are developing a SiPM-based DOI detector named X’tal cube to achieve sub-mm spatial resolution, which is reaching the theoretical limitation of PET imaging. We have developed a prototype for 1mm isotropic detector resolution, which equals the world record.

2) Quantitative analyses of in vivo imaging

The Imaging Physiology Team develops methods for quantitative analyses of in vivo imaging obtained from PET imaging, MRI, and optical imaging. In PET receptor imaging, a new graphic plot analysis was evaluated for a reliable quantification of binding potential, and it was shown that this could provide unbiased binding potentials. In MR diffusion-tensor imaging, an oscillating-gradient spin-echo sequence to diffusion-tensor imaging was applied to in vivo rat brain, and pixel-wise linear fits to the mean diffusivity found elevated changes across the cerebellum. Using laser-Doppler flowmetry, we found that the increase in red blood cell velocity during sensory stimulation was much larger than that of concentration in awake mice, supporting the PET measurement of CBF and CBV during neural activation in humans. An analytical method to quantitatively measure vessel diameters and flow dynamics was developed for fluorescent confocal microscopic imaging, and this method revealed that cortical surface vascular tone and parenchymal blood flow were coordinated in rat brain. In two-photon laser scanning fluorescence microscopy, the redistributed parenchymal microcirculation among the capillary networks induced by brain activation was demonstrated with gene manipulated rats in which red blood cells express green-fluorescent proteins.

Diagnostic Imaging Program

1) Basic clinical research on pathophysiological imaging with molecular imaging

We have conducted clinical PET research using \( ^{18}F \)-fluorothyridine, a marker of cellular proliferation, in lung cancer and malignant melanoma patients receiving carbon-ion radiotherapy and proved its role as a prognostic indicator. As a model of carcinogenesis, we have established a facilitated model of radiation-induced thymic lymphoma in mice and are applying various molecular imaging methods for imaging the process of lymphoma development. We have developed an efficient production method of an amino acid PET probe, \( ^{11}C \)-AIB, and proved that \( ^{11}C \)-AIB has a potential in differentiating cancer from inflammation (Fig.3). For the biological characterization of cancers that are refractory to treatment, we are focusing on tumor hypoxia, and clinical PET research using a hypoxia PET probe, \( ^{18}F \)-FAZA, is ongoing with regard to its ability to predict the responsiveness to treatment and prognosis, along with basic preclinical research for the biological characterization of intra-tumoral areas showing high accumulation of hypoxia PET probes.

2) Development of antibody and peptide probes for the targeted imaging of various cancer-related molecules

In research on the imaging of cancer molecular targets, we have developed antibody probes targeting pancreatic cancer-related molecules and successfully carried out PET imaging of subcutaneously and orthotopically transplanted pancreatic cancer in mice by using \( ^{64}Zr \)-labeled human monoclonal antibody recognizing transferrin receptor. We have also developed a PET probe to visualize cancer angiogenesis, a tetramer of cRGD peptide labeled with \( ^{64}Cu \), targeting integrin \( \alpha \beta \) expressed on activated endothelial cells, and proved that this probe could image cancer angiogenesis and also could evaluate the effect of anti-angiogenesis therapy (see Highlight for detail).

3) Development of MRI-based functional probes and nano-sized multi-functional probes and their application in various disease models

In research on the application of MRI-based functional probes, we have shown that the change in cellular uptake of manganese (Mn) after irradiation in Mn-enhanced MRI was related to radiation-induced alteration in the cell cycle. We have also developed an MRI probe to measure tissue redox status and applied it to
healthy and cancer-bearing mice for depiction of the changes in tissue redox status according to the disease condition (see High-light for detail). We have developed and improved a nano-sized drug-delivery system (nano-DDS) using liposomes coated with thermo-sensitive polymer containing anti-cancer agent, fluorescent dye and MRI contrast agent, and succeeded in getting efficient drug release by local heating that could be imaged by MRI; we also proved the treatment effect after combined nano-DDS, local heating and irradiation.

Molecular Neuroimaging Program

There are three major research targets of the Molecular Neuroimaging Program: the development and evaluation of imaging biomarkers of several types of dementia in the key process of their pathophysiology; the investigation of molecular mechanisms of the symptoms related to regional brain functions using both clinical data and model animal experiments; and, the development of surrogate imaging markers for evaluation of treatment of neuropsychiatric disorders.

1) Imaging biomarker of dementia

In the field of development and evaluation of imaging biomarkers of several types of dementia, we have found serotonin 1A receptor change in tau transgenic mice, metabotropic glutamate receptor change in amyloid precursor protein transgenic mice, and translocator protein (TSPO) change in mild cognitive impairment (MCI) patients. Those neurotransmission changes in model mice indicated that a possible target for the treatment of dementia and the TSPO change in MCI patients might indicate a possible involvement of an inflammation process even in early stage of dementia. The most important result in this field is the development of a tau imaging probe. We have successfully evaluated it in Alzheimer’s disease (AD) brain; the accumulation of our tau probe was negligible in healthy control brain but significant accumulation was observed in the hippocampal region of AD brain where the accumulation of [11C]PiB was relatively low compared to other cortical regions. Furthermore, our preliminary data suggested that our tau probe may be capable of capturing the temporospatial spreading of neurofibrillary tau pathologies from the transentorhinal cortex to other limbic and neocortical association areas with the progression of AD.

2) Molecular mechanism of regional brain functions

Regarding the mechanisms of human behavior, which has long been investigated by various disciplines including philosophy, psychology, economics, and biology, we have used functional MRI (fMRI) to investigate regional brain function mechanisms and PET to investigate molecular mechanisms. We provide unique neurobiological evidence to account for individual differences of reaction to unfairness; higher central serotonin transmission might allow humans to behave adroitly and opportunistically, being good at playing games while pursuing self-interest. We also elucidated neural circuits for mitigating criminal sentences. Individual differences on the inclination to mitigate, the sentence reduction per unit of judged sympathy, correlated with activity in the right middle insula, an area known to represent interception of visceral states. These results could help the legal system understand how potential jurors actually reach a decision and they could contribute to the growing body of knowledge about whether emotion and cognition are integrated sensibly in difficult judgments. Combining both fMRI and PET, we found an interrelationship between dopamine neurotransmission and fronto-striatal resting-state functional connectivity in the basis for cognitive bias such as a “superiority illusion”. To clarify the direct molecular and physiological mechanisms of emotion and behavior, experiments with an animal model are quite important. Using a monkey model of hypothyroidism, which is associated with symptoms of low motivation characterized by instrumental task performance, we found the performance of goal-directed action was affected by dopaminergic manipulations. PET revealed that there was a change in dopamine D2 receptor in the ventromedial prefrontal cortex of the model monkey. The combination of different imaging techniques is essential in modern neuroscience to unite cognitive neuroscience and neuropsychopharmacology.

3) Surrogate imaging markers in therapy

In neuropsychopharmacology, various target molecules and the kinetics of drugs targeting them can be visualized using PET. We have examined dopamine receptor occupancy of antipsychotics and serotonin transporter occupancy of antidepressants. Another important target of antidepressants is norepinephrine transporter (NET). We have measured NET occupancy of 75-200 mg/day of nortriptyline and found approximately 50-70% occupancies in the living human brain.
Objectives

Glutamate is an excitatory neurotransmitter in the central nervous system (CNS) and plays a role in neurotransmission via activation of its receptors e.g. ionotropic and metabotropic (mGlu) types. MGlur receptors are classified into three groups including eight subtypes according to sequence homology, coupling mechanisms to G-protein, and pharmacological activity. Group I of the mGlu receptors (mGlu1 and mGlu5) plays important physiological roles in regulating ion channels and synaptic transmission, and in synaptic plasticity, which underlies memory and learning. It has been reported that mGlu1 may be a drug target for the treatment of diseases such as stroke, epilepsy, pain, cerebellar ataxia, Parkinson’s disease, anxiety, and mood disorders. To elucidate the role of mGlu1 for these diseases, many PET ligands have been developed for mGlu1. Recently, we developed \( ^{18}F \)FITM as a novel PET ligand for imaging mGlu1 in the brain. To find a PET ligand with more favorable in vivo behavior, we designed various candidates using \( ^{11}C \)ITMM as a lead compound. In this report, we summarize our published findings on synthesis and evaluation of \( ^{11}C \)ITMM and its \( ^{18}F \)fluoroalkylated derivatives as new PET ligands for mGlu1 in the brain.

Overview

First, as shown in Fig.1A, the novel compounds ITMM and fluoroalkylated derivatives 2 and 3 were synthesized at 5-7 steps from 4,6-dichloropyrimidine. \( ^{11}C \)ITMM was prepared by reaction of desmethyl precursor 1 with \( ^{11}C \)CH\(_3\)I of two levels of specific activity (37-185 GBq/\( \mu \)mol and 3700-7400 GBq/\( \mu \)mol) at 70°C for 5 min in the presence of NaOH (Fig.1B). Two \( ^{18}F \)fluoroalkoxy ligands \( ^{18}F \)2 and \( ^{18}F \)3 were synthesized by reaction of 1 with \( ^{18}F \)FEtBr and \( ^{18}F \)FPrBr, respectively, at 90°C or 120°C for 10 min.

In vitro binding affinities of ITMM, 2, and 3 for mGlu1 were measured from competition against the binding of mGlu1-selective \( ^{11}C \)FITM using rat brain homogenates. Among these ligands, ITMM showed the highest binding affinity (K\(_i\) = 12.6 nM) for mGlu1. On the other hand, fluoroopropyl 3 displayed the lowest affinity (K\(_i\) > 5 \( \mu \)M). This result suggested that the bulk-increasing group attached to the 4-position of the benzene ring may not fit the binding site on the mGlu1 domain.

The lipophilicities (Log D) for \( ^{11}C \)ITMM, \( ^{18}F \)2, and \( ^{18}F \)3 were 2.57-2.80 measured by the shake flask method and their values were found in the range normally considered favorable for PET.

In vitro autoradiography with \( ^{11}C \)ITMM, \( ^{18}F \)2, and \( ^{18}F \)3, as for \( ^{11}C \)ITMM, the distribution pattern of radioactivity was heterogeneous, with the highest level in the cerebellum. Moderate radioactivity was seen in the thalamus and a low level was seen in the striatum. This result was consistent with the distribution pattern of mGlu1 in the rat brain. Co-incubation with mGlu1-selective JNJ-16250685 reduced radioactivity in the sections to 30% of the ra-
dioactivity in control sections. Thus, we found that \[^{11}\text{C}]\text{ITMM}\] showed high specificity for mGlu1 in vitro. In the case of \[^{18}\text{F}]\text{2} and \[^{18}\text{F}]\text{3}, both showed much lower radioactivity in the brain than \[^{11}\text{C}]\text{ITMM} did. Moreover, co-incubation with JNJ-16259685 did not affect radioactivity in the brain. Therefore, we selected only \[^{11}\text{C}]\text{ITMM}\] for in vivo evaluation.

The in vivo uptake, kinetics, and specific binding in the rat brains were examined using small-animal PET with \[^{11}\text{C}]\text{ITMM}\] of 95-140 GBq/μmol. \[^{11}\text{C}]\text{ITMM}\] showed high brain penetration and accumulation of radioactivity in the brain regions as shown in Fig. 2. The highest uptake was seen in the cerebellum, followed by the thalamus, and striatum. The lowest radioactivity was determined in the pons. This distribution pattern of uptake reflected the distribution of mGlu1 in the brain\(^{10}\), which was similar to that in vitro autoradiograms for \[^{11}\text{C}]\text{ITMM}. Regarding the kinetics, radioactivity in the cerebellum gradually increased after injection, peaked at 45 min, and decreased to 90% of the maximum at 90 min. Radioactivity in the thalamus and striatum peaked also at 45 min and declined slowly until the end of the PET scan. Pretreatment with unlabeled ITMM or JNJ-16259685 markedly reduced the uptake compared to the control.

Furthermore, PET scanning with \[^{11}\text{C}]\text{ITMM}\] was performed in mGlu1 knockout mice to confirm the specificity of \[^{11}\text{C}]\text{ITMM}\] for mGlu1. In the wild-type mouse, accumulation of radioactivity was seen in the cerebellum and thalamus, which was similar to the accumulation seen in PET images of rat brains. On the other hand, in the mGlu1-knockout mouse, only a very low radioactivity was determined in the brain. The present results indicate that \[^{11}\text{C}]\text{ITMM}\] is a promising PET ligand for mGlu1. Currently, \[^{11}\text{C}]\text{ITMM}\] as the first useful PET ligand for mGlu1 is undergoing clinical trials in the human brain.

**References**


Objectives

Metallic radionuclides play a prominent role in both diagnostic nuclear medicine and internal radiotherapy. While we, as the Radiopharmaceutical Production Team, have been providing many kinds of high quality metallic radionuclides routinely, we are also developing novel, cost-effective, and automated production methods for these radionuclides. To accommodate both routine production and research under the condition of limited beam time, easy operability with less effort and time or a least-waste production method is highly desirable.

Therefore, our research goal is not only achievement of products at high yield and with high quality, but also satisfying practical engineering needs; namely promoting efficiency of the production process from preparation to final purification and establishing remote production methods with less or no radiation exposure at low cost.

Overview

In the production of metallic radionuclides, using a conventional cyclotron or an irradiation system with a horizontal beam line is a laborious process and it takes a relatively long time to prepare the solid target, if secured metal foils or plates are unavailable commercially. One of the objectives in this research is to reduce the number of such time-consuming processes by applying a vertical irradiation system that produces radionuclides by using a downward transported beam. A target material, which is the source for the intended radionuclide, can be held in place easily by gravity with this system. This irradiation mechanism provides the great benefit of being able to use almost any form of chemical substance as the target material. Namely, non-self-supported materials, such as metal powders, granules, low melting point substances, or salts can be placed at the beam trajectory without the need for a prior solidification process. Thus, the time for target preparation is greatly reduced.

Remote handling of the highly radioactive material is another issue to be resolved. Briefly, the irradiated target should be removed remotely from the beam port, and then the target will be transferred to a hot cell for further processing. A versatile industrial robot, custom made remote-arms, or transporting cart on rails is usually employed in conventional remote production systems. The purification process is then carried out by the following steps: i) disassembling the target vessel to take out the target material; ii) dissolving the irradiated target by strong acids; iii) isolating and purifying the intended radionuclide from other nuclides, especially those in the target material and impurities. These steps are also carried out remotely by using a manipulator or specialized device to reduce radiation exposure. As mentioned above, the handling of an “immobile” solid target, which is a counterpart of the “mobile” gaseous and liquid target, would need hand-or-foot-like external driving forces. However, such devices are large-scale heavy systems that generally increase installation and maintenance costs, and occupy a sizable working place; therefore only a few facilities are able to accommodate them.

In this research, we demonstrated a simple and cost-effective remote production method by introducing strong acids into the target vessel to obtain the radio-metal solution in situ. The intended nuclide in liquid form should be easily transferable to the hot cell through a tube by applying appropriate pressure without using any huge robotic devices. However, conventional materials used for target vessels are metals, such as Al, Ti, or stainless steel, which are less durability against acids. Therefore, we designed new target vessels made of ceramics, namely alumina (Al₂O₃) or silicon carbide (SiC), to demonstrate the concept of in situ target dissolution. Fortunately, both ceramics have favorable properties, such as excellent chemical resistance, fair thermal conductivity, and they are not molten in a practical temperature range, which are essential requirements for the target vessel materials.

We evaluated the feasibility of the ceramic target vessels with the vertical irradiation system for production of zirconium-89. ⁸⁹Zr is one of the most promising positron emitters applicable to...
immuno-PET studies due to its relatively long half life of 78 h, and it can be produced by a relatively low beam energy from yttrium-89 via the \( ^{89}Y(p,n)^{89}Zr \) nuclear reaction. Fortunately, the natural isotopic abundance of \(^{89}Y\) is 100%; this makes it favorable for studies at modest cost, and the result can be obtained at high accuracy with less waste. Briefly, instead of secured Y foils, \(^{89}Y\) powder prepared in the ceramic target vessel was employed as the target material. Under this condition, we evaluated the following items:

1) irradiation of a powder target while keeping the product yield at a sufficient level;
2) dissolution of the irradiated target remotely in the ceramic vessel by introducing an acid solution;
3) transfer of the radioactive solution to the hot cell through a tube automatically; and
4) capability to repeat the production while keeping the system integrity.

An automated apparatus for the purification of \(^{89}Zr\) was also developed in this study (Fig.2). This apparatus design put emphasis on the production of highly concentrated \(^{89}Zr\) by including reagent reservoirs and an ion-exchange column to purify the \(^{89}Zr\) efficiently. All separation steps including the target-dissolving step were carried out remotely and automatically via a PC-based controller.

The integrity of the ceramic target vessel was kept while repeating the remote productions more than 10 times. The yield of \(^{89}Zr\) with >99.9% radionuclide purity obtained by this method was about 90% of the expected value calculated from a previous report about the excitation function\(^{[3]}\). The processing time for each production run was typically within 2.5 h. The product \(^{89}Zr\) of 925 MBq in 90 μL of oxalic acid, obtained by 10 μA x 2 h irradiation, was successfully provided to immuno-PET studies. The irradiation of the powder target gave a successful result, and it was confirmed some of the laborious target preparation processes could be eliminated. Therefore, all of the objectives in this research were fully achieved.

**Conclusion**

Although we used Y powder as a target material in this pilot study, the production scheme is, in principle, applicable to other metal targets including isotopically enriched materials. Furthermore, the recovery process from the target vessel was similar to that of gaseous and liquid target production using the ceramic vessel. This means that the remote production of metallic radionuclides becomes accessible to many facilities with less effort and at lower cost.

Indeed, we have started to produce other metallic radionuclides by a modified method based on this result, and we strongly believe that novel production methods for medically important radionuclides will be shown in the near future.

**Further expectations**

The vertical irradiation technique is currently recognized as a specialized one because most cyclotrons or beam lines are designed to provide horizontal beams. However, we believe that a medical compact cyclotron with upright dee plates has inherent potential to deliver downward beams with minimal reconfiguration. By remodeling the magnetic fields and/or the position of the electron stripper, a downward or vertical beam can be obtained without using a huge bending magnet or extensive modification.

In the future, the development and distribution of cyclotrons equipped with vertical beam ports, used in combination with the ceramic target vessel developed here, are expected to make production of metallic radionuclides and associated applications more convenient and popular.

**References**


We developed a novel, general purpose isotropic-3D PET detector X’tal cube which has high spatial resolution in all three dimensions. The research challenge for this detector was implementing effective detection of scintillation photons by covering six faces of a segmented crystal block with photo-detectors. Also, in order to fabricate the 3D crystal block efficiently and precisely, we applied a laser-processing technique to a monolithic crystal block instead of gluing segmented small crystals. Using the fabricated X’tal cubes, we evaluated its imaging resolution performance to show a proof-of-concept of isotropic resolution.

Typical PET detectors are designed with a 2D array of segmented scintillator crystals that are coupled to photomultiplier tubes on one side. However the parallax error caused by the thickness of the crystals degrades spatial resolution at the peripheral regions of the field-of-view (FOV). Therefore, depth-of-interaction (DOI) measurement is essential to achieve high spatial resolution. The X’tal cube \cite{1, 2} is our original PET detector, which is being developed to achieve isotropic 3D positioning detectability.

The X’tal cube is based on a 3D segmented crystal block for which all surfaces are covered with photo-detectors (Fig.1 (a)). Instead of our initial approach of gluing segmented pieces of crystals, we successfully constructed a crystal block segmented by laser processing \cite{3}, and we developed the first prototype of X’tal cube with the laser-processed 3D square grids of 2 mm length. Also, we extended the laser processing to 3D square grids of 1 mm length (Fig.1 (b)). The volume of a 1-mm crystal segment is 1/8 of that of a 2-mm crystal segment. We also evaluated imaging resolution performance with a newly developed one-pair prototype system to simulate a ring-type scanner. The one-pair prototype system consisted of two X’tal cubes, two rotating stages, and...
a 192-channel data acquisition system (Fig.2). Each X’tal cube consisted of the LYSO cubic crystal block of (18 mm)$^3$ in which the 3D square grids of 1 mm length were fabricated by internal laser processing. The $4 \times 4$ arrays of multi pixel photon counters (MPPCs) were optically coupled to each surface of the crystal block. The detector positions were automatically controlled to simulate a ring-type PET with a 14.6 cm diameter. Data were collected for all assumed detector positions and then a sinogram was obtained. The data were reconstructed using filtered back-projection. Fig.3(a) shows the 3D position histogram of the 1-mm X’tal cube obtained from the 511-keV uniform irradiation. Also, each spot corresponded to a 3D grid. Almost all the 3D grids on the 3D position histogram could be separated clearly. Fig.3(b) shows radial and tangential full width at half maximum (FWHM) resolutions for the 1-mm X’tal cube. Without DOI information, the spatial resolutions were degraded at off-center positions. The average spatial resolution of the 1-mm X’tal cube was 1.3 mm FWHM over the FOV. By applying deconvolution with the assumption that the point source was a Gaussian function of 1.0 mm FWHM, we estimated the average spatial resolution of the 1-mm X’tal cube as 0.83 mm FWHM. In conclusion, we confirmed the potential of the X’tal cube for uniform and high resolution imaging.

References


Fig.2 Photographs of: (a) the one-pair prototype system and (b) the X’tal cube. (c) Schematic illustration of the virtual ring-type PET scanner with a 14.6 cm diameter.

Fig.3 (a) 3D position histograms from the 511 keV uniform irradiation of the 1-mm X’tal cube and (b) radial and tangential FWHM resolutions for the 1-mm X’tal cube.
Positron emission tomography has been utilized for imaging neuroreceptors in the human brain. Because quantitative analysis of receptor binding potential (BP) usually requires 60-min PET scanning, head-movement correction is necessary for a reliable quantification. In the present study, a system for image-based motion correction was developed, and an optimal correction method was evaluated using a computer simulation and human data of \(^{11}\)Craclopride-PET.

Positron emission tomography (PET) can visualize receptor binding in living human brains. In this PET measurement for quantifying receptor binding potential (BP), a transmission scan is performed before administration of tracer to obtain the \(\mu\)-map used for the attenuation correction, and after the tracer administration, a 60-90 min interval of consecutive emission data is acquired to obtain the time course of accumulated radioactivity. Therefore, head movement is often observed during the emission scanning, especially in the later frames, and it hampers reliable quantification. To correct head movement, in general, image-based or hardware-based motion correction is applied\(^{1-3}\). In the image-based motion correction, information on head movement among time frames is computed by coregistering each frame of a reconstructed emission image to a reference frame image. Unlike hardware-based motion correction, this method is easy to implement and does not require an online tracking system. However, the reliability of frame-by-frame coregistration depends on the distribution of the radioisotope in emission images, signal-to-noise ratio, the reference image, and so on. Therefore, it is important to evaluate an optimal coregistration method according to specific administered tracers.

In addition, frame-to-frame realignment of emission images causes a mismatch between the emission and transmission images, and it may result in error of the quantitative outcomes. In the present study, we developed a system for image-based motion correction in PET receptor imaging, and we evaluated an optimal method of image coregistration for PET studies with \(^{11}\)Craclopride by a computer simulation. Then, this methodology was applied to PET studies with \(^{11}\)Craclopride of normal volunteers, and the effect of this correction on quantitative analysis outcomes was investigated.

First, we constructed a data processing system for image reconstruction including head-movement correction for PET data acquired with Eminence SET-3000GCT/X (Shimadzu Corp., Kyoto, Japan). In this system, motion-corrected images are generated as follows (Fig.1). (1) A transformation matrix representing motion among time frames is calculated from automatic frame-by-frame coregistration using a reconstructed emission image. (2) A \(\mu\)-map obtained through transmission scan is resliced using the transformation matrix so that its coordinate matches to the coordinate of each time frame of the emission image. (3) In the measured emission sinogram, that is a set of projection data of the administered tracer in the brain, attenuation is corrected frame-by-frame using attenuation data derived from forward-projection of the resliced \(\mu\)-map. (4) Radioactivity image of each time frame is reconstructed by a filtered-back projection from the attenuation-corrected emission sinogram. (5) Each frame of the reconstructed radioactivity image is realigned to the coordinate of the first frame image using the transformation matrix.

Next, an optimal method of frame-by-frame image coregistration for estimating the transformation matrix was evaluated by a computer simulation. The emission sinogram that imitated human \(^{11}\)Craclopride-PET data with translation or rotation head movement was simulated, and reconstructed with or without attenuation correction. Each frame of these reconstructed images was automatically coregistered, using mutual information, to various reference images, such as the PET summation image of all frames, an early frame image, a high-count frame image, and a previous frame image. The reliability of coregistration was evaluated by comparing the estimated transformation matrix with true values. As a result, reconstructed images without attenuation correction could be coregistered precisely to early and high-count frame images.
Finally, the image-based motion correction was applied to PET studies with $^{11}$C raclopride of normal volunteers. After the correction, reconstructed images were frame-to-frame realigned correctly (Fig.2), and discontinuity of time-activity curve in the striatum was mitigated (Fig.3). The binding potential estimated by a simplified reference tissue model became larger when obvious head movement was observed in the later frames.

In summary, head movement during a PET dynamic scan was accurately corrected by applying the optimal frame-by-frame coregistration and reconstruction with the resliced $\mu$-map, and it remarkably improved the reliability of quantitative outcomes. This method is practical for clinical research, because it does not require a hardware system for online motion tracking and can be applied to all PET data, such as previous data acquired without motion tracking.

References


Background and objectives

Angiogenesis, the formation of new blood vessels from pre-existing vasculature, in a tumor is a key feature of malignant solid tumors, plays a critical role in tumor growth, invasion, and metastasis, and has been accepted as an important target and indicator of therapeutic outcome and prognosis. \(\alpha_\beta\) Integrin, one of the key biomarkers for tumor angiogenesis, is a transmembrane glycoprotein receptor and highly expressed on activated endothelial cells during angiogenesis. Cyclic pentapeptides containing a tripeptide sequence RGD, cRGDs, are optimized synthetic ligands that have a high affinity and selectivity for \(\alpha_\beta\) integrin. RAFT-c(-RGDfK-)4, designed and developed by Pascal Dumy and colleagues of Joseph Fourier University, is a tetrameric cRGD-containing peptide that is synthesized by separately grafting 4 cRGD motifs onto the upper side of the cyclic decapeptide platform called RAFT (regioselectively addressable functionalized template) to form the \(\alpha_\beta\)-targeting domain. On the lower side of RAFT, a variety of substances such as fluorescent dye, radioisotope or peptide can be linked to form the functional domain.

In collaboration with Dr. Dumy’s group, we have developed a novel RAFT-c(-RGDfK-)4 based positron emission tomography (PET) probe for noninvasive visualization and quantification of tumor angiogenesis and monitoring of antiangiogenic efficacy via targeting the tumor \(\alpha_\beta\) integrin [1-3], as introduced in the following parts.

Major research results in FY 2011 for the development of \(^{64}\text{Cu}-\text{cyclam-RAFT-c(-RGDfK)-}4\)

1) Synthesis of cyclam-RAFT-c(-RGDfK)-4 and radiolabeling with \(^{64}\text{Cu}\)

RAFT-c(-RGDfK)-4 was prepared through a combination of solid and solution-phase syntheses, and was conjugated with cyclam, a bifunctional chelator, to form a molecule (molecular weight \(\sim 5\) kDa) for \(^{64}\text{Cu}\)-labeling (Fig.1). The radiolabeling procedure for cyclam-RAFT-c(-RGDfK)-4 is easy, mild, and straightforward. In brief, the peptide solution and \(^{64}\text{CuCl}_2\) reconstituted in ammonium citrate buffer were mixed and incubated at 37°C within 1 h. The radiolabeling efficiency for cyclam-RAFT-c(-RGDfK)-4 was >99%, and the specific radioactivity that could be achieved was as high as \(~37\) MBq/nmol.

2) In vitro and in vivo studies for determining the binding activity and specificity of \(^{64}\text{Cu}-\text{cyclam-RAFT-c(-RGDfK)-}4\) for \(\alpha_\beta\) integrin

In vitro binding studies showed much stronger binding of \(^{64}\text{Cu}-\text{cyclam-RAFT-c(-RGDfK)-}4\) for \(\alpha_\beta\)-overexpressing cells than for \(\alpha_\beta\)-negative cells, demonstrating its \(\alpha_\beta\)-binding activity and specificity. The \(\alpha_\beta\) specificity was further confirmed by the dose-dependent competitive binding inhibition using the \(\alpha_\beta\)-specific c(RGDfV) peptide. Compared to c(RGDfV), cyclam-RAFT-c(-RGDfK)-4 showed a much higher affinity or avidity for \(\alpha_\beta\), as shown by comparing their IC50 values (39 nM for cyclam-RAFT-c(-RGDfK)-4 versus \(~2642\) nM for c(RGDfV)).

Biodistribution studies demonstrated that \(^{64}\text{Cu}-\text{cyclam-RAFT-c(-RGDfK)-}4\) had a rapid blood clearance, predominant renal excretion pathway, low-level uptake in nontumor tissues, and high tumor-to-background contrast. The tumor-targeting specificity of \(^{64}\text{Cu}-\text{cyclam-RAFT-c(-RGDfK)-}4\) was confirmed by the blocking study.

3) Correlation of tumor \(\alpha_\beta\) integrin expression with tumor uptake of \(^{64}\text{Cu}-\text{cyclam-RAFT-c(-RGDfK)-}4\)

For determining the correlation, biodistribution assay was performed to measure the \(^{64}\text{Cu}-\text{cyclam-RAFT-c(-RGDfK)-}4\) uptake values for various tumors, and the \(\alpha_\beta\) expression levels of these tumors were then quantified by SDS-PAGE/autoradiography. A linear and positive correlation was found between the \(\alpha_\beta\) expression and the tumor radioactivity accumulation of \(^{64}\text{Cu}-\text{cyclam-RAFT-c(-RGDfK)-}4\).
4) PET imaging of mice bearing tumors with different levels of $\alpha\beta_3$ integrin

$^{64}$Cu-cyclam-RAFT-c(-RGDfK-)$_4$ PET scans were finally performed in mice bearing tumors with different expression levels of $\alpha\beta_3$. As compared to HEK293(β1) tumor with undetectable levels of $\alpha\beta_3$, HEK293(β3) (high levels of $\alpha\beta_3$) and U87MG tumors (moderate levels of $\alpha\beta_3$) were clearly visualized with high contrast relative to the contralateral background at all the time points of 1–20 h p.i. The highest radioactivity accumulation in the tumor was visualized at 1 h p.i., and this was followed by a gradual washout with time. Fig. 2 clearly shows a high, a moderate and a weak tumor uptake of $^{64}$Cu-cyclam-RAFT-c(-RGDfK-)$_4$ for HEK293(β3), U87MG and HEK293(β1) tumors, respectively, corresponding to their $\alpha\beta_3$ expression levels. Overall, the PET images agreed well with the biodistribution data.

Major research results in FY 2012 for the application of $^{64}$Cu-labeled cyclam-RAFT-c(-RGDfK-)$_4$ for tumor angiogenesis study

1) Use of HuH-7 xenograft as a tumor angiogenesis model and PET imaging of tumor angiogenesis

We studied angiogenesis in a tumor xenograft derived from the $\alpha\beta_3$-negative human hepatocellular carcinoma HuH-7 cell line to eliminate interference from $\alpha\beta_3$ integrin expressed on the tumor cells themselves. Our study proves that $^{64}$Cu-cyclam-RAFT-c(-RGDfK-)$_4$ PET enabled visualization of tumor angiogenesis by targeting $\alpha\beta_3$ integrin. The imaging quality was good because the tumors could be clearly visualized at both 1 and 3 h p.i., which was supported by the biodistribution study showing high tumor-to-blood and tumor-to-muscle ratios of ~32 and ~7, respectively, at 3 h p.i. In addition, no correlation was found between tumor weight and tumor uptake (expressed as %ID/g, a percentage of injected dose per gram of tissue) of $^{64}$Cu-cyclam-RAFT-c(-RGDfK-)$_4$, indicating that the tumor size itself is not a critical factor influencing tracer uptake.

2) Antiangiogenic efficacy as assessed and evaluated by PET imaging

Administration of the antiangiogenic drug TSU-68 (75 mg kg$^{-1}$ d$^{-1}$, i.p.) in HuH-7 tumor-bearing mice for 2 weeks resulted in retardation in tumor growth and reduction in tumor microvessel density (MVD) determined by CD31 immunostaining. The results obtained from the same set of experiments showed that the TSU-68-induced reduction in tumor MVD was accompanied by a reduction in the tumor standardized uptake value (SUV) determined by $^{64}$Cu-cyclam-RAFT-c(-RGDfK-)$_4$ PET. Moreover, a positive and significant correlation was found between the tumor MVD and the corresponding SUV (either the mean or maximum value) or %ID/g evaluated by biodistribution assay. Representative PET images from the vehicle and TSU-68-treated mice acquired at 3 h p.i. of $^{64}$Cu-cyclam-RAFT-c(-RGDfK-)$_4$, are shown in Fig. 3. Visually, the radioactivity was obviously lower in the tumors from the TSU-68-treated mice than in those from the controls. Further, while the radioactivity accumulated in the control tumors was homogeneous, the radioactivity signals in the TSU-68-treated tumors were heterogeneous. Autoradiographic examination and immunofluorescence staining (Fig. 3) demonstrated the intratumoral colocalization of the tracer and vascular network distribution in which murine β3 integrin was found positive. Taken together, our results strongly demonstrate that the antiangiogenic effects of TSU-68 can be monitored by quantitative $^{64}$Cu-cyclam-RAFT-c(-RGDfK-)$_4$ PET imaging.
Fig. 2  PET imaging of mice bearing subcutaneous HEK293(β3), U87MG or HEK293(β1) tumors at 3 h after i.v. injection of 11.1 MBq 64Cu-cyclam-RAFT-c(-RGDfK-)4. Arrows indicate tumor localization.

Fig. 3  PET images of mice bearing subcutaneous HuH-7 tumor at 3 h after i.v. injection of 11.1 MBq 64Cu-cyclam-RAFT-c(-RGDfK-)4 on day 15 after treatment with vehicle or TSU-68. Arrows indicate tumor localization. After PET imaging, tumors were excised, and autoradiographic examination and CD31 immunofluorescence staining were performed with the same whole-tumor sections.
Summary and prospectus

We have developed a PET probe $^{64}$Cu-cyclam-RAFT-c(-RGDfK-)$^4$ that exhibited high binding affinity and specificity for the $\alpha_v\beta_3$ integrin, and favorable pharmacokinetics. Positive linear correlation was observed between tumor uptake of $^{64}$Cu-cyclam-RAFT-c(-RGDfK-)$^4$ and tumor $\alpha_v\beta_3$ levels, which was also revealed by the noninvasive PET imaging study. We used murine xenografts from an $\alpha_v\beta_3$-negative tumor cell line and showed that $^{64}$Cu-cyclam-RAFT-c(-RGDfK-)$^4$ PET enables the clear visualization of tumor angiogenesis and helps monitor the effectiveness of antiangiogenic therapy. In future, we intend to determine whether this strategy is effective for tumors in which $\alpha_v\beta_3$ is expressed on both tumor cells and the neovasculature by using longitudinal PET imaging to detect not only changes in tracer uptake but also changes in the tracer distribution pattern. Further, it may also be applicable for monitoring angiogenic therapy in other angiogenesis-associated disorders such as ischemia, atherosclerosis, and myocardial infarction. Finally, because $^{64}$Cu also emits $\beta^-$, the application of $^{64}$Cu-cyclam-RAFT-c(-RGDfK-)$^4$ for internal radiotherapy to increase therapeutic gain should also be investigated.

References

Abstract

Experiences in free radical biology and medicine have shown the crucial role of redox signalling in carcinogenesis. The cells and tissues of healthy mammals are characterized by a low level of reactive oxygen species (ROS) and some constant (reference) level of reducing equivalents. Increasing the ROS above the critical level provokes genomic instability and normal cells become malignant.

The present study describes a universal methodology for direct imaging of tissue redox activity in carcinogenesis on intact animals; the method allows a differentiation of cancer development from the normal condition. Experiments were conducted on cancer-bearing mice (grafted with neuroblastoma, glioma or colon cancer) and healthy mice as controls. The tissue redox activity was visualized in vivo by nitroxide-enhanced MRI on anesthetized animals or in situ by EPR spectroscopy on isolated tissue and blood specimens. The method is based on the nitroxide redox cycle, coupled with appearance and disappearance of MRI/EPR signal. The half-life ($\tau_{1/2}$) of the nitroxide-enhanced MRI signal in the respective tissue was used as a diagnostic marker. The study provided direct evidence that healthy and cancer-bearing mammalian tissues were characterized by different redox activity — a basis for a cancer diagnostic term. The tissues (cancer and “normal”) of cancer-bearing mammals were characterized by a longer-lived MRI signal, a decrease of total antioxidant capacity, and an increase of matrix metalloproteinases (MMP2 and MMP9) relative to controls, indicating a higher oxidative activity. The tissues of healthy organisms were characterized by a shorter-lived MRI signal and a higher total antioxidant capacity, indicating a high reducing activity.

An important observation is that the oxidative status of non-cancer tissues of cancer-bearing organisms (even far from the primary tumour locus) increases with cancer progression and they become susceptible to oxidative stress and damage. The non-cancer tissues also have to be considered as a therapeutic target. The study directly relates to the cancer diagnosis and assessment of cancer progression, using molecular imaging, as well as to the therapeutic planning strategy. Since, the tissue redox status is very sensitive to radiotherapy and chemotherapy, the proposed methodology can be used for assessment of therapeutic effects in dynamics using molecular imaging. The method is simple and applicable on isolated tissue and blood specimens. It has a real potential to be applied for in situ and in vivo imaging diagnosis on humans after development of cell-penetrating nitroxide probes with high contrast, low toxicity and minimal side effects.

Introduction

Over 50 years of experiences in free radical biology and medicine have shown the crucial role of redox signalling in carcinogenesis\cite{1}. The cells and tissues of healthy mammals are characterized by a low steady-state level of oxidizers (e.g., reactive oxygen species (ROS) and reactive nitrogen species (RNS)) and some constant (reference) level of reducers (e.g., endogenous redox pairs: NADH/NAD$^+$, NADPH/NADP$^+$, FADH$_2$/FAD, reduced/oxidized glutathione, reduced/oxidized ascorbate, etc.). It is widely accepted that increasing the ROS/RNS above the critical level provokes genomic instability and triggers uncontrolled proliferation. The normal cells become malignant.

Cancer cells are characterized by an abnormal production of reducing equivalents as a result of accelerated glycolysis (Warburg effect) and the pentose phosphate cycle, but also by a rapid consumption of these reducers to maintain accelerated anabolism, which is necessary for cell proliferation and immortalization. Cancer cells need also a lot of antioxidants to maintain ROS/RNS below the threshold level, above which apoptosis and cell death are induced, but this level has to be high enough to ensure genomic instability. These processes provoke redox imbalance in cancer and this parameter can be used as a diagnostic marker, a therapeutic target, and a hallmark for evaluation and planning of the therapeutic strategy.
There is no universal non-invasive methodology for estimation of tissue redox activity in intact mammals. The oxidizing and/or reducing status of tissues are determined by the levels of many parameters (e.g., ROS/RNS of different types and origins, products of free radical oxidation of biomacromolecules, status of natural non-enzymatic and enzymatic antioxidant systems, status of various endogenous redox pairs, etc.). Each parameter is analysed separately by different methodologies in vitro or in situ. The estimation of redox status of cancer and healthy tissues is based on comparative analysis of one or several of these parameters and the conclusions are usually controversial.

We propose an approach for direct imaging of tissue redox activity in vivo on intact healthy and cancer-bearing mammals, which allows a differentiation of cancer development from the normal (healthy) condition. The method is based on the redox cycle of cell-penetrating nitroxide derivatives and their MRI (magnetic resonance imaging) contrast properties, which makes them useful molecular sensors for tissue redox activity (Fig.1). The nitroxide radical (which is characterized by T1 contrast) participates in electron-transfer reactions with oxidizers and reducers with formation of contrast or non-contrast intermediate products [2]. The rate constants of these reactions determine the intensity of the nitroxide-enhanced MRI signal in living cells and tissues. In healthy mammals, the half-life of the nitroxide-enhanced MRI signal (τ1/2) in the selected region of interest (ROI) is considered as a reference value of tissue redox activity in the normal condition (healthy organism). We established that in the same or similar ROI of cancer-bearing mammalian tissues, τ1/2 was completely different from the reference value and this parameter is a valuable diagnostic marker for carcinogenesis.

Nitroxide radical can also be characterized by electron paramagnetic resonance (EPR) spectroscopy, allowing determination of the exact concentration and redox status of nitroxide derivative in cancer or non-cancer tissues. The comparative analysis of the results, obtained by both imaging techniques, gives accurate information about tissue redox activity in vivo and in situ.

**Experimental**

In our study, we used cell- and blood-brain barrier (BBB)-penetrating nitroxide with DNA-annealing and anti-cancer effects — nitroxide-labelled nitrosourea (SLENU), for MR imaging of tissue redox activity in healthy and cancer-bearing mice (grafted with neuroblastoma, glioma or colon cancer). The aims of the study were to examine: (i) which of the two processes — oxidation or reduction, dominates in cancer and non-cancer tissues, using a single measurement on intact mammals; (ii) whether the nitroxide-enhanced MRI is suitable for cancer diagnosis in various cancer models; and (iii) what are the potential molecular mechanisms underlying redox signalling in carcinogenesis.

All experiments were conducted in accordance with the guidelines of the Physiological Society of Japan and were approved by the Animal Care and Use Committee of NIRS.

Several experimental schemes were used: (i) comparison of tissue redox activity between healthy and cancer-bearing mice; (ii) comparison of tissue redox activity between cancer-bearing mice in different stages of cancer development (early, intermediate and terminal); (iii) investigation of the molecular mechanisms underlying redox signalling in carcinogenesis — from early to terminal stages of cancer, including the role of metalloproteinasies, total antioxidant capacity, reactive oxygen species and integrin signalling cascade.

To distinguish the tissues of healthy mice from those of cancer-bearing mice, we used the following terminology: normal tissue — tissues of healthy mice (controls); “normal” tissue — non-cancer tissues of cancer-bearing mice; and cancer tissue — tissue in the cancer area, which is visualized structurally by MRI. Representative figures are presented below. Details are described in our recently published studies [2,3].

**Results**

Fig.2A shows typical kinetics of MRI signal intensity in the brain (ROI1) and surrounding tissues (ROI2) of healthy mice after injection of nitroxide. In both ROI, the signal increased after injection, followed by rapid decrease to the baseline. The half-life of nitroxide-enhanced MRI signal (τ1/2) was ~80 s. This value can be considered as a reference for the redox activity of both tissues at normal conditions (e.g., healthy mice). The increase of the MRI signal in the beginning was due to the presence of nitroxide in the blood and its penetration and accumulation in the subsequent tissue, while the rapid decrease was due to its reduction to non-contrast hydroxylamine in the cells. This was confirmed by EPR spectroscopy on isolated tissue specimens. This profile of the histograms indicated a high reducing activity of normal (healthy) tissues to the nitroxide radical.

In neuroblastoma-bearing mice (in the terminal stage of cancer), the kinetics of the MRI signal intensity in both ROIs after in-
Injection of SLENU was completely different from the reference profiles (Fig. 2B). In ROI1, the signal increased after injection and reached a plateau without decreasing within 14 min ($\tau_{1/2} \gg 14$ min).

In ROI2, the signal increased after injection, then decreased slowly without reaching the baseline within 14 min ($\tau_{1/2} \approx 14$ min).

These histograms indicated a high oxidative activity of the cancer tissue and surrounding "normal" tissues of cancer-bearing mice. This was also confirmed by the pharmacodynamics and redox status of nitroxide in the brain, detected by EPR spectroscopy on isolated tissue specimens.

The kinetic curves of the MRI signal had the same profiles in both hemispheres — cancer-bearing and "normal" (Fig. 2C), however the signal intensity was significantly higher in the cancer area (Fig. 2D). This was direct evidence about the higher oxidative activity of cancer tissue in comparison with "normal" tissues of cancer-bearing organism and tissues of healthy organism.

Similar results were obtained on glioma-bearing and colon-cancer bearing mice in the terminal stage of cancer [9]. The data from biochemical analyses showed an enhancement of the plasma matrix metalloproetinases (MMP2 and MMP9), a decrease of tissue total antioxidant capacity, and an activation of integrin-signalling cascade in cancer-bearing mice, in comparison with healthy mice (Fig. 3).

**Conclusion**

In conclusion, the study shows that tissue redox activity can be used as a sensing platform for molecular imaging of cancer by nitroxide-enhanced MRI and cell-penetrating nitroxides. We give
direct proof in vivo that the tissues of cancer-bearing mammals are characterized by high oxidative activity, while the tissues of healthy organisms are characterized by high reducing activity to the nitroxide. The high oxidative activity of cancer tissue is a fact despite hypoxia development in solid tumours. It relates to the abnormal production of ROS/RNS, but not to high oxygen tension. This is in agreement with the widely-accepted opinion that cancer cells are characterized by increased production of ROS/RNS relative to normal cells, which ensures genomic instability[1].

The most significant observation is that the oxidative status of non-cancer tissues of cancer-bearing organisms (even far from the primary tumour locus) increases with cancer progression and they become susceptible to oxidative stress and damage. This finding shows that it is necessary to develop a more tolerant and efficient therapeutic strategy. In this context, combining anticancer therapy with protection of non-cancer tissues against oxidative stress could be essential for survival and recovery of the organism. Since tissue redox status is very sensitive to radiotherapy and chemotherapy, the proposed methodology can be used for dynamic assessment of therapeutic effects using molecular imaging (MRI/EPR).

The present study has a high translational relevance. It is directly related to cancer diagnosis, assessment of cancer progression, and planning of therapeutic strategy. It shows that the tissue redox balance is very sensitive to the cancer development and can be used as a hallmark of carcinogenesis. The method is simple and applicable to isolated tissue and blood specimens. We think it has a real potential for future applications to in vivo imaging diagnosis in humans.

References
The evolution of cognitive neuroscience has been largely driven by the development of quantitative measures of human brain function (e.g., fMRI). This methodological advancement has enabled researchers to address hypotheses that were previously inconceivable, such as a causal relationship between patterns of neural activity, cognitive processes and complex human behavior. In addition to the quest for localization of function, another approach to study the human mind has been nurtured by examining molecular mechanisms, particularly in clinical domains. An emerging emphasis is now being placed on integrating multiple levels of neural responses from a molecular system to a neural response/circuit - to produce adaptive behavior, paralleling the social world in which humans live.

Integration of different disciplines has also borne fruit in the field of cognitive neuroscience by the convergence of perspectives (e.g., neuroscience, social psychology, economics, and ethology). This new wave of research has moved past basic work aimed at understanding brain function toward examining the neural underpinnings of issues critically important to humanity. This is admittedly a lofty goal, but cognitive neuroscience has already made significant contributions across several spheres of society, given by an illuminating example of our study, which is the integration of cognitive neuroscience and the law.

Philosophers, psychologists and legal scholars have long debated whether mercy, sympathy and compassion should reduce moral culpability of defendants in criminal cases. People have negative emotional responses to a wide range of situational factors that are not normatively justifiable legally. Social and moral neurosciences provide converging evidence of the interplay between negative emotion and moral judgments (e.g., a trolley dilemma), but the influence of sympathy on legal decision-making is unknown. The legal domain is unusual because it may be especially challenging to map emotions into numerical legal outcomes. Uncovering the cognitive and neural mechanisms of sympathy that motivate mitigation will inform the role of emotion in the jurors’ decision process, and what role emotional evidence can and should play in trials.

We measured brain activity using functional MRI while subjects were making hypothetical sentence reduction decisions in dramatic scenarios adapted from actual murder cases. Mitigating circumstances were of two types: Those that would induce sympathy, and those that would not. The sympathy scenarios included desperate situations of defendants suffering from domestic violence, disease, or poverty. After reading about the circumstances, subjects decided how much they would change the sentence given for the defendant (initially 20 years) if they were on a jury (Fig.1A). After scanning, subjects were again presented with the same scenarios and asked to rate how much sympathy they felt for the defendant. Behavioral performance confirmed the internal validity of the sympathy manipulation (Fig.1B).

We first searched for brain regions that responded, during the description, to the subjects’ trial-by-trial ratings of sympathy and their amounts of punishment reduction. Activity in the precuneus, dorsomedial prefrontal cortex (DMPFC) and left temporo-parietal junction (TPJ) were correlated with sympathy (P < 0.05, small-volume-corrected; Fig.1C). Signal increase in the precuneus, DMPFC and anterior cingulate cortex (ACC) were also associated with the reduction of punishment (P < 0.05, small-volume-corrected, Fig.1C).

The DMPFC is involved in general mentalizing and is active when empathizing with others in pain. The precuneus, dorsomedial prefrontal cortex (DMPFC) and left temporo-parietal junction (TPJ) were correlated with sympathy (P < 0.05, small-volume-corrected; Fig.1C). Signal increase in the precuneus, DMPFC and anterior cingulate cortex (ACC) were also associated with the reduction of punishment (P < 0.05, small-volume-corrected, Fig.1C).

The DMPFC is involved in general mentalizing and is active when empathizing with others in pain. The precuneus has been linked to subjective perspective taking. The TPJ is also commonly identified as a part of the theory-of-mind circuit and was activated in one study on judging innocence of intentions. This suggests that the sympathy judgment is an engagement with a reasoned simulation of what the defendant was thinking when committing the crime or how most people would judge the normative basis for mitigation. Precuneus activation is also correlated with more iterated steps and higher-value strategic thinking in game theory.
tasks. The overlapping precuneus activity between a feeling of sympathy and judged mitigation of punishment suggests that the precuneus may be a region that accepts emotional judgment input, and maps it into concrete punishment actions. Activations in the ACC and caudate are interpreted as conflict resolution and pro-social choices, respectively, which are associated with mitigating behavior.

We note that one fMRI study reports right DLPFC activity associated with responsibility judgments. The absence of DLPFC activation in our study is possibly because there is no doubt about the defendants’ guilt, so the most morally burdensome question of guilt versus innocence is resolved (the DLPFC is discharged from jury duty, so to speak).

Next, we constructed an individual-specific measure of an inclination to mitigate, by reducing sentences, as a function of sympathy. The b1 coefficient of the regression in punishment = b0 + b1*sympathy + error represents a complex mapping from an emotional response to a number representing prison time for a defendant (a years-per-emotion coefficient).

A negative linear regression between the individual-specific b1 coefficient and BOLD responses in sympathy minus no-sympathy trials found activity in the right middle insula (P < 0.05, small-volume-corrected, Fig. 2). Individuals who had larger activities in the insula when reading circumstances showed higher tendencies to mitigate, reducing sentencing years more as their sympathy increased. The middle or posterior insula has been linked to interoceptive processing in various social tasks (e.g., inequity), which suggests this area is sensitive to emotions linked to sociality. Our study provides unusual evidence of this processing associated with a unique high-impact social judgment that affects others.

Taken together, the identified brain activity is encouraging about the capacity of the average brain to translate sympathetic feelings into appropriate legal action. Activity in these “sympathy” regions is evident in our study when judging sympathy alone, and in choosing sentence mitigation. However, not every brain maps sympathy to prison sentences in the same numerical way (as reflected in differential mid-insula activity). Differences represent a legal challenge about how to tolerate and weigh differential juror responses. There is also mixed evidence about the normative basis of legal judgment, including a recent finding that judges’ decisions are affected by timing of meals.

Finally, we note that many legal principles treat emotional responses as likely to be prejudicial and prone to inflammatory manipulation (i.e., an ideal juror would suppress them, and legal rules limit their influence). Weighing mitigating circumstances during sentencing (after a verdict) represents an unusual case in which emotional sympathy judgment is actually required. Ironically, the fact that sympathy is clearly evident in brain activity, and influences sentence mitigation (as it should), raises interest in the opposite question: Can people also suspend emotions when the law instructs them to? More generally, a deeper understanding of the brain could help figure out how highly-evolved brain structures, which were sculpted to maintain order in small-scale ancestral societies, can be put to work under modern legal rules in much more challenging cases, to create modern justice.

References

Introduction

Dementia with Lewy bodies (DLB) and Parkinson’s disease with dementia (PDD) are categorized as belonging to the same spectrum, Lewy body disease with dementia. In addition to pathognomonic Lewy body pathology, DLB/PDD frequently has Alzheimer’s disease (AD)-type pathology, particularly amyloid beta (Aβ) plaque. The contribution of Aβ to the development of DLB/PDD remains unclear.

More than half of all DLB patients, and about one-third of all PDD patients had a cortical Aβ burden in previous studies using [11C]PIB-PET. Meanwhile, previous MRI volumetric studies suggested that DLB/PDD patients showed the AD-like cortical atrophy in several brain regions including the parahippocampal area. It is still unclear, however, whether the AD-like brain atrophy observed in DLB/PDD is associated with Aβ burden or not.

To elucidate this issue, we compared the grey matter volume measured by voxel-based morphometry (VBM) among DLB/PDD patients with and without cortical Aβ burden, AD patients, and healthy control subjects.

Methods

1) Subjects

Participants were 8 patients with DLB, 7 patients with PDD, 13 patients with AD, and 22 healthy controls (HC) diagnosed as PIB-negative (PIB(-)) by visual assessment of distribution volume ratio (DVR) images of [11C]PIB PET (Table 1).

2) PET and MR images acquisition

A dose of [11C]PIB was intravenously injected and sequential PET scans were performed for 90 min by Siemens ECAT EXACT HR+ scanner.

Subjects were also scanned with a 3D T1-weighted turbo gradient echo sequence on a Philips 1.5T Intera.

3) PET data preprocessing

All imaging data were preprocessed and analyzed with SPM5. We estimated DVR using Logan plot graphical analysis with the cerebellum as the reference region. VOIs were identified on DVR images in each subject’s frontal, medial and lateral temporal, parietal, occipital, anterior and posterior cingulate and sensorimotor cortices and striatum in both hemispheres using the Wake Forest University (WFU) PickAtlas.

Subjects with significantly increased DVR greater than or equal to mean + 2.5 SD of the HC group in at least one brain region were classified as PIB(+), and subjects with no significantly increased PIB uptake in any brain region were classified as PIB(-). Furthermore, voxel-based analysis of DVR among the HC, PIB(-) and PIB(+) DLB/PDD, and AD groups was performed.

4) Voxel-based morphometry

We performed VBM analysis using the unified segmentation approach implemented in SPM5 for each 3D T1-weighted MR image. VOIs were identified on modulated, normalized and warped class images using the WFU PickAtlas.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographic and neuropsychological test results of participants</th>
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</table>

Values are listed as mean±SD.

**Results**

1) **PET**

All HCs were PIB(-), while all AD patients, half (4/8) of the DLB patients and 29% (2/7) of the PDD patients were PIB(+). There were no significant differences between the PIB(-) and PIB(+) DLB/PDD groups in respect to any clinical profiles.

All PIB(+) DLB/PDD patients showed a similar distribution pattern of increased DVR in the brain to AD patients (Fig.1A).

2) **Voxel-based morphometry**

SPM analysis showed more profound cortical atrophy in both the AD and PIB(+)DLB/PDD groups than in the HC group especially in the temporal (including parahippocampus) and parietal areas (including precuneus) (Fig.1B), whereas the PIB(-) DLB/PDD group did not show significant cortical atrophy. The brain regions where grey matter volume was significantly reduced compared to the HC group in the PIB(-) and PIB(+) DLB/PDD groups overlapped with those in the AD group by 95.2% and 0%, respectively.

VOI analysis (Table 2) revealed that, compared with the HC group, parahippocampal grey matter volumes were reduced in both the PIB(+) DLB/PDD (Z score = 1.94 ± 0.60, p = 0.002, 25.8% reduction) and AD (1.91 ± 0.78, p < 0.001, 25.5% reduction) groups, whereas those in the PIB(-) DLB/PDD group did not differ from the HC group (0.76 ± 0.60, p = 1.000, 10.2% reduction). Furthermore, in the AD and PIB(+) DLB/PDD groups, significant grey matter volume reduction compared to controls was shown in frontal, parietal, occipital and lateral temporal cortices, striatum, hippocampus and amygdala; while there was no significant grey matter reduction in the PIB(-) DLB/PDD group.

**Discussion**

The present study demonstrated that DLB/PDD patients with high cortical PIB uptake had AD-like cortical atrophy in the parahippocampal area, lateral temporal and parietal cortices compared to the HC subjects. Furthermore, the DLB/PDD patients with high cortical PIB uptake showed parahippocampal atrophy similarly to the AD patients, as compared to the DLB/PDD patients with low cortical PIB uptake. These results suggest that Aβ deposition is associated with AD-like atrophy in DLB/PDD patients.

In previous reports, the patterns of cortical atrophy observed in DLB and PDD were controversial. The different results of previous studies could be explained by the difference in the prevalence of subjects with high cortical Aβ deposition.

A pathological study by Foster et al. reported that a high cortical Aβ score along with an older age at onset were associated with a shorter time-to-dementia period in PDD. Rowe et al. reported that cortical PIB-binding was correlated inversely with the interval from onset of cognitive impairment to diagnosis in DLB. Longitudinal studies in DLB/PDD patients will be required to elucidate whether Aβ deposition accelerates the progression of dementia and brain atrophy, and whether α-synuclein accelerates Aβ deposition in the brain.

It may perhaps be a more interesting finding in this study that there was no striking grey matter atrophy despite the presence of dementia in PIB(-) DLB/PDD patients. Graff-Radford et al. reported that patients with DLB but without the imaging features of coexistent AD-related pathology, such as parahippocampal atrophy and PIB(+), were more likely to cognitively improve with acetylcholinesterase (AChE) inhibitor treatment. These findings would suggest a predominant subcortical mechanism, such as cholinergic dysfunction, underlying the dementia in the beta-amyloid negative Lewy body patients with dementia.

In conclusion, our results suggest that Aβ deposition is associated with AD-like atrophy in DLB/PDD. Early intervention against Aβ may prevent or delay AD-like atrophy in patients with DLB/PDD with Aβ deposition.

**References**


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Table 2. Reduction of grey matter volumes; values were normalized to total intracranial volumes compared to controls Mov Disord, 28(2), 169-75, 2013.

<table>
<thead>
<tr>
<th></th>
<th>DLB/PDD</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIB(-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIB(+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIB(+) DLB/PDD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIB(-) DLB/PDD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal</td>
<td>1.65 ± 1.54</td>
<td>2.60 ± 1.40</td>
</tr>
<tr>
<td>Sensorimotor</td>
<td>0.85 ± 1.15</td>
<td>1.22 ± 1.05</td>
</tr>
<tr>
<td>Parietal</td>
<td>1.34 ± 1.22</td>
<td>2.08 ± 1.30</td>
</tr>
<tr>
<td>Striatum</td>
<td>1.22 ± 1.30</td>
<td>2.14 ± 2.06</td>
</tr>
<tr>
<td>Occipital</td>
<td>1.18 ± 0.86</td>
<td>1.94 ± 1.04</td>
</tr>
<tr>
<td>Lateral temporal</td>
<td>1.40 ± 0.93</td>
<td>3.22 ± 1.24</td>
</tr>
<tr>
<td>Parahippocampal area</td>
<td>0.76 ± 0.50</td>
<td>1.91 ± 0.78</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.66 ± 1.07</td>
<td>2.21 ± 0.96</td>
</tr>
<tr>
<td>Amygdala</td>
<td>0.83 ± 1.43</td>
<td>3.71 ± 1.59</td>
</tr>
</tbody>
</table>

Grey matter volume reduction compared to controls adjusted by each intracranial volume was expressed by Z score, and values are listed as mean ± SD.

a: HC vs PIB(-) DLB/PDD; b: HC vs PIB(+ DLB/PDD; c: HC vs AD; d: PIB(-) DLB/PDD vs AD; e: PIB(+) DLB/PDD vs PIB(+ DLB/PDD. *p < 0.05, **p < 0.01, t p < 0.005, f p < 0.001 (ANOVA, adjusting for differences in age, followed by Bonferroni correction)