Iwao Kanno, Ph. D.
Director, Molecular Imaging Center

(Outline of Research Activities)
Iwao Kanno started his professional career at Akita Research Institute of Brain and Blood Vessels in 1970, where he was an active researcher for 36 years. In 1997, he developed a custom radionuclide emission tomography system using a handmade rotational dentist chair. In 1979 he developed a hybrid type of emission tomography which combined positron emission tomography (PET) and single photon emission computed tomography (SPECT). His efforts were also directed to developing methodology for quantitative assessment of physiological and biochemical parameters from PET and SPECT images. In 1997, he developed a custom radionuclide emission tomography system using a handmade rotational dentist chair. In 2006, he joined NIRS where he continues his research career.
Objectives

Progress in molecular biology has opened the window to understanding the molecular mechanisms of living healthy and diseased organs. Molecular imaging is a new interdisciplinary field that integrates imaging technology and molecular biology to help visualize molecular behaviors spanning the microscopic to macroscopic scales. PET, magnetic resonance imaging (MRI) and optical imaging will provide clear and comprehensive images demonstrating molecular functions. The Molecular Imaging Center consists of four research groups, Diagnostic Imaging Group, Molecular Neuroimaging Group, Molecular Probe Group and Biophysics Group, and the Research Promotion Unit. The Molecular Imaging Center aims to image molecular functions of living animals in both healthy and diseased conditions. Of several methodologies for imaging molecular functions, the center covers in vivo molecular imaging from rodents to humans. It is already a world leader in the development of PET probes and technologies, and it also has invested efforts in other promising technologies such as MRI. Our primary goals are to move towards understanding the mechanism of brain function and cancer pathology and to use this knowledge in clinical applications.

Overview

The Diagnostic Imaging Group continued our clinical PET study with FLT, a marker of cell proliferation, in the evaluation of cancer patients receiving carbon ion radiotherapy in collaboration with the Research Center for Charged Particle Therapy. A multi-center study of PET with 68Ga-ATSM, a marker of tumor hypoxia, is also being conducted. To identify novel targets of mesothelioma, functional screening using siRNA was carried out, which newly identified at least 7 genes having anti-apoptotic function. One of these genes is now being investigated as a target of siRNA-based treatment of malignant mesothelioma. We also found that cellular contents of Mn and Mn-SOD are increased in various mesothelioma cells, suggesting the biological significance of Mn in mesothelioma. We then attempted the visualization of mesothelioma using Mn-MRI, which is giving promising results. Research on the development of an antibody probe for cancer imaging was continued using anti-c-Kit and anti-ER/HR/ERG monoclonal antibodies. For the application to PET imaging, a labeling method with 111In was optimized and biodistribution of radiolabeled Fab fragments was evaluated. Research on PET imaging of cancer neovascularization using 111In-labeled RGD peptide is also ongoing. A novel probe to detect the activated state of EGFR was designed, which showed specific retention in cancer cells with EGFR activation. A rat model of syngeneic and allogeneic liver transplant was established and FDG-PET was proven to be useful not only in the detection of acute allograft rejection but also in the evaluation of immunosuppressive treatment.

The Molecular Neuroimaging Group established PET quantification methods using a dopamine D2 receptor agonistic probe, [11C]MPPA and a peripheral benzodiazepine receptor (PBR) probe, [11C]AC-5216. The inverted U-shaped relation between the prefrontal dopamine D1 receptor and the cognitive function was found in healthy subjects. The functional MRI study showed that the emotion of “envy” was regarded as a psychological pain as suggested by the activated regions. Clinical PET studies with patients demonstrated the increase in the striatal k1 of [11C]DOPA in schizophrenics and the widespread accumulation of [11C]DA1106 in the brain of Alzheimer's disease sufferers. PET protocols calculating the D2 receptor occupancy to evaluate therapeutic effects of antipsychotics have been optimized. An in vitro imaging analysis of mice modeling a psychotic state revealed prominent changes in levels of monoamine neuroreceptors and transporters. A PET study of awake rats and monkeys elucidated the localization of metabotropic glutamate receptors involved in the regulation of the striatal dopamine release. Two newly developed 18F radioligands for amyloid plaques and some imaging agents of fibrillar tau were evaluated by in vivo imaging tools accompanied with model mice and they are expected to be useful for clinical diagnoses. Utilization of our original materials for PBR delineated that PBR could be upregulated among the gliosis in the brain of Alzheimer's disease model mice and confirmed the correlation between levels of PBR and glial cell line-derived neurotrophic factor in activated astrocytes. Brain regions relating to the addictive cocaine intake in monkeys were identified using H11C PET, although no changes of dopamine D1 and D2 receptors in extrastriatal regions have been found. Modeling of Parkinson's disease using MPTP-treated marmosets was established and the prominent loss in dopamine transporters by means of [11C]PE2I PET was demonstrated.

The Molecular Probe Group has been developing novel molecular probes for PET and SPECT imaging. We developed a method for assessing multidrug resistance-associated protein 1 (MRP1) function in vivo using PET and a newly developed PET probe, 6-halo-7-[11C]methylpurine. When the molecular probe was administrated to Mrp1 knockout mice, the efflux rate of the radioactivity was reduced to approximately 90% compared with wild-type mice. This is the world's first method which allows noninvasive and quantitative assessment for exporter function in the living brain. We also evaluated [11C]DAC as a novel PET ligand and for
imaging of PBR in kainic acid-lesioned rat brain. A small-animal PET study determined that $^{[11]C}$DAC had high uptake in the lesioned region, where PBR density was increased. The high in vivo specific binding of $^{[11]C}$DAC to PBR is available as a new biomarker for brain injuries, neuroinflammations, and tumors, etc. Moreover, gefitinib was synthesized and used for tumor imaging and evaluation of P-gp/BCRP function. In vivo distribution study on NFSa-bearing mice revealed that $^{[1]C}$gefitinib specifically accumulated into the tumor. A PET experiment produced a clear tumor image in mice. It was demonstrated that the brain penetration of $^{[1]C}$gefitinib was related to both P-gp and BCRP. In order to develop a new labeling method, $^{[1]N}$N-acetyl chloride and $^{[1]C}$nitromethane were applied for the synthesis of $^{[1]N}$urea, $^{[11]C}$oseltamivir and 2-Amino[2-$^{[15]C}$]ethanol. A new synthesis apparatus which supports synthesis of $^{[1]C}$oseltamivir from a preparation of $^{[1]C}$acetyl chloride and using the $^{[1]C}$acetylation reaction and subsequent deprotection reaction, was developed. The apparatus can produce $^{[1]C}$oseltamivir in sufficient yield and quality for animal PET studies. A rapid and efficient preparative high-performance liquid chromatographic procedure utilizing a hydrophilic interaction chromatography column and a highly volatile organic mobile phase was established to purify short-lived PET probes. Four new PET probes ([$^{[1]C}$]Ac5216, [$^{[15]F}$]TO-002, [$^{[1]C}$]gefitinib and [$^{[15]F}$]FAZA) were approved by the Institutional Review Board at NIRS and released for clinical research.

The Biophysics Group consists of four teams. The Magnetic Resonance Molecular Imaging Team investigated: therapeutic drug delivery imaging using a temperature-sensitive liposome; a multimodal nanoprobe using quantum-dots; detection of reactive gliosis; immunocyto labeling and tracking; and a multimodal therapeutic contrast agent using nitroxy radical. The Biosignal Physiology Team investigated: diffusion functional MRI; MR elastography for clinical use; human studies using evidence-based molecular imaging; direct visualization with fluorescent microscopy; and intracortical microcirculation visualized with multi-photon microscopy. The Image Analysis Team developed algorithms and experimental apparatuses to measure and visualize various functionalities of humans and animals using PET, and also developed a system for arterial sampling from mice in which the allowed amount of sampled blood is 1 μL. The Imaging Physics Team proposed an improved OpenPET geometry. The OpenPET mainly has three applications, namely, simultaneous PET/CT, extension of the axial FOV, and in-beam PET, which is a method for in situ and non-invasive monitoring of tumor-conforming charged particle therapy. This team also proposed a new depth-of-interaction (DOI) PET detector design, which was named “Xtal cube”.

Recently, small, light, and thin photodetectors such as avalanche photodiodes (APDs) or multi-pixel photon counters (MPFCs) have become commercially available as alternatives to photomultiplier tubes (PMTs). In this design, therefore, a number of the small photodetectors are coupled to a 3-dimensional scintillation crystal array at any six surfaces.
4.1. Research on Molecular Imaging of Cancer

Tsunco Saga, Ph.D.
Director, Diagnostic Imaging Group

[Outline of Research Career]

Dr. Saga received a Ph.D. from Kyoto University in 1991 for his investigations on cancer targeting of radiolabeled monoclonal antibodies. He continued his research on antibody targeting at the National Institutes of Health (1991-1993) and at Kyoto University (1995-2006). In addition, for the last 9 years, he has been conducting clinical and basic research covering the wide area of cancer imaging. Since 2006, he has been the leader of the Diagnostic Imaging Group at NIRS; this group works to further advance the basic and clinical research on molecular imaging of cancers.

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Objectives

The Diagnostic Imaging Group is conducting research on functional imaging of cancer by PET and other modalities. By using various cancer-specific probes, the characteristics of an individual cancer such as malignant grade and responsiveness to treatment can be clarified. This information can be used for treatment planning and evaluation of therapeutic effect. Although several PET probes such as FDG and $^{11}$C-methionine are available for clinical studies, development of new imaging probes is necessary for more comprehensive evaluation of cancers and to further contribute to the management of cancer patients.

The Clinical Diagnosis Team focuses on clinical research on oncological PET and is aiming to contribute to the management of cancer patients including those considered for carbon ion radiotherapy (CIRT) conducted in the Hospital of the Research Center for Charged Particle Therapy. In addition to FDG and $^{11}$C-methionine, we are evaluating newly developed cancer-imaging probes, such as $^{18}$F-FLT and $^{65}$Cu-ATSM, to determine their clinical usefulness.

The Molecular Diagnosis Team conducts basic molecular imaging research focusing on designing and evaluation of PET probes that capture and depict the changes of biomolecules specifically associated with cancers and other diseases to realize effective non-invasive diagnoses. We also develop novel in vivo reporter gene imaging systems to facilitate the establishment of new therapies such as gene therapy and regenerative therapy.

The Biomolecule Team focuses on elucidating genetic/molecular events occurring during carcinogenesis and searching for suitable targets of molecular imaging of cancers. By using functional screening of genes and proteome analysis of the blood and tissue samples, we select the genes and proteins specifically expressed in cancers. Through the exploration of the targets with high specificity, we are aiming for the development of novel molecular imaging methods which can depict the characters of each cancer.

Progress in Research

1) Clinical studies on cancer imaging using various PET probes

We are conducting clinical PET research using FLT, a marker of cell proliferation, in the evaluation of effectiveness of CIRT. Data from more than 20 lung cancer patients showed that the development of radiation pneumonitis after CIRT modified tumor FLT uptake and made precise post-treatment evaluation difficult. More importantly, patients who developed recurrence/metastasis showed significantly higher pre-CIRT FLT uptake than patients who did not develop recurrence/metastasis.(Fig. 4-1)

Fig. 4-1 Comparison of pre-CIRT FLT uptake with the development of recurrence/metastasis

We are also conducting PET with $^{64}$Cu-ATSM, a marker of tumor hypoxia, for cancer patients receiving CIRT. The uptake pattern of $^{64}$Cu-ATSM varied from that of $^{11}$C-methionine probably reflecting the difference in distribution of tumor hypoxia and amino acid metabolism within the tumor tissue. Comparison with the treatment response is ongoing.

2) Loss of function screening to identify therapeutic and diagnostic targets in malignant mesothelioma

Malignant mesothelioma is a highly aggressive tumor arising from serosal surfaces of the pleura. To identify therapeutic and/or imaging molecular targets, we conducted a large-scale functional screening of mesothelioma cells using small interfering RNAs (siRNAs) against 8,589 human genes. We determined that knockdown of 39 genes apparently suppressed mesothelioma cell proliferation. At least seven of these 39 genes would be involved in an anti-apoptotic function. One of them was highly expressed in some mesothelioma cell lines, but not in a normal mesothelial cell line. Knockdown of this gene using siRNAs induced apoptosis and suppressed tumor growth not only in vitro but also in vivo. This gene would be useful for developing effective therapeutic agents of mesothelioma.

4) Development of animal models to facilitate the development of imaging probes and therapies

In the development of cancer imaging probes and therapy strategies, model animals play crucial roles. Other than mouse models, we have developed a fluorescent cancer model in “Medaka”. The GFP (green fluorescent protein) expressing tumor cells were grown at the injection site, and the spatiotemporal changes were visualized under a fluorescence stereoscopic microscope with a cellular level-resolution,
even at a single cell level. Tumor dormancy and metastasis were also observed. Our Medaka model provides a new opportunity to visualize in vivo tumor cells “as seen in a culture dish” and is useful for in vivo tumor cell biology and facilitates the development of cancer imaging probes and therapeutics.

5) Development of PET/SPECT tumor imaging using antibody probes

For the imaging of c-kit-positive tumors such as gastrointestinal stromal tumor, we labeled anti-c-kit monoclonal antibodies (MAbs) (IgG and Fab) with single-photon ($^{111}\text{In}$) and positron ($^{68}\text{Cu}$) emitters, and assessed their in vitro and in vivo characteristics. The radiolabeled MAbs showed specific binding to c-kit-expressing cancer cells and $^{111}\text{In}$-labeled IgG and $^{68}\text{Cu}$-labeled Fab highly accumulated in xenografted tumors which were clearly visualized by SPECT and PET.

To image epithelioid mesothelioma, radiolabeled MAbs (IgG and Fab) recognizing the mesothelioma related antigen (ERC/mesothelin) was also assessed. The radiolabeled MAbs specifically bound to mesothelioma cells and were internalized after binding. $^{111}\text{In}$-labeled IgG and $^{68}\text{Cu}$-labeled Fab accumulated in xenografted tumors which were readily visualized by SPECT and PET.

6) Development of novel reporter gene imaging

In order to develop a novel reporter gene imaging, we are evaluating a ferritin heavy chain (FHC) gene as a reporter. In vitro experiments demonstrated that cells transiently expressing FHC gene showed increased cellular uptake of iron resulting in the decreased T2 weighted (T2W) MR signal. When the plasmid designed to express FHC gene together with RFP (red fluorescent protein) was electroporated into mouse subcutaneous tumor, a localized region of lowered T2W signal was observed which coincided with the region of RFP expression. Now we are exploring the possible application of this reporter gene, including a model stably expressing FHC.

7) Search for specific molecular target of mesothelioma imaging and its visualization

During the extensive search for specific molecular targets of mesothelioma imaging, we found that the contents of heavy metals, such as manganese (Mn) and copper (Cu), in various mesothelioma cell lines, are increased compared to normal mesothelial cells indicating the possibility that these heavy metals are involved in mesothelioma formation and/or progression. The Mn content in each cell line was especially well correlated with Mn-SOD expression, suggesting the biological significance of Mn in mesothelioma cells. We then attempted the visualization of mesothelioma using Mn-MRI. MRI imaging of the mesothelioma cells expressing a high level of Mn-SOD gave enhanced MRI images in vitro and the in vivo MRI imaging of xenografts of the cells are giving promising results as well.

8) Development of neovascularization and tumor imaging by PET

Tumor neovascularization is important not only in the local growth of tumors, but also in tumor invasion and metastasis. Integrin $\alpha$, $\beta$, is expressed on the surface of endothelial cells of newly formed vessels in tumors and on some tumor cells. Various analogs of RGD peptides bind to integrins and have been used for imaging tumor neovascularure. Among them, RAFT-c(RGD), which was developed by Dr. Dumy and contains 4 cyclic RGDs in a single molecule (RGD tetramer) is a very specific and high affinity ligand for integrin $\alpha$, $\beta$, . In collaboration with Dr. Dumy's group, we have synthesized cyclam conjugated RAFT-c(RGD), which can be labeled with positron emitting Cu isotopes and used for PET imaging. The labeling with Cu-64 was very efficient and the radiochemical purity was over 90% without purification. The initial small animal PET imaging of integrin $\alpha$, $\beta$, overexpressing tumor gave clear visualization of the tumor.

9) Development of PET probes for EGFR imaging

EGFR (epidermal growth factor receptor) is often overexpressed and/or mutated in many cancer cells and its abnormal activation is implicated in carcinogenesis and cancer progression. To characterize the cancer and aid treatment planning, we attempted to develop imaging probes to capture the activated state of EGFR. We designed a peptide probe binding to activated EGFR based on the SH2 domain of Grb2, adding TAT for delivery into cells and tissues. In vitro experiments confirmed the uptake of the peptide probe into the cells leading to the binding to EGFR. Now we are exploring possible use of the probe in vivo.

10) $^{18}$F-FDG-PET of acute allograft rejection and therapy efficacy in liver transplantation rat models

Acute liver allograft rejection remains a major complication after liver transplantation. We developed a semi-quantitative imaging method of detecting acute allograft rejection using $^{18}$F-FDG-PET. We established syngeneic and allogeneic liver transplantation models in rats. $^{18}$F-FDG uptake significantly increased in liver allografts on day 2 and further increased thereafter. Histopathological study on day 3 exhibited moderate rejection of the allografts. Autoradiography showed that $^{18}$F-FDG signals were concentrated in the area where inflammatory cells aggregated around the vessels. Administration of immunosuppressive agents prevented the increase in hepatic $^{18}$F-FDG uptake (Fig.
4-2). 

"F-FDG-PET imaging would be a valid method for the diagnosis of graft rejection and also for the monitoring of immunosuppressive therapy.

Fig.4-2. Detection of acute rejection and the effect of immunosuppression by FDG-PET

11) CIRT efficacy in mouse model of malignant mesothelioma

Since the prognosis of patients with malignant mesothelioma with current multimodality therapy remains poor, it is important to develop a new and more effective treatment. To assess the efficacy of CIRT for mesothelioma, we evaluated its effect in epithelioid and sarcomatoid mesothelioma mouse models. Both epithelioid and sarcomatoid tumor xenografts irradiated with 15-Gy carbon ion irradiation apparently regressed. We have conducted further experiments at higher doses of carbon ions or X-rays, and are measuring PET tracer uptake in tumors after irradiation to explore the correlation of treatment effect and the uptake of PET tracers.

**Major publications**


4.2. Molecular Neuroimaging Research

Tetsuya Suhara, M.D., Ph.D.
Director, Molecular Neuroimaging Group

(Outline of Research Career)

Dr. Suhara received the Ph.D. from Jikei University School of Medicine in 1991 for his study of dopamine receptor binding in vivo. He began working at NIRS in 1989. From 1992-1993, he studied in the PET group of the Department of Clinical Neuroscience, Karolinska Hospital, Sweden. He has researched brain functional imaging for many years. He has served as a visiting professor at the Department of Neuropsychiatry, Nippon Medical School from 2004, and at the Graduate School of Medicine, Yokohama City University from 2006.

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Objectives

1) Clinical Neuroimaging
   b) Explore the relation between the regional dopaminergic neurotransmission functions and the higher brain functions in healthy human subjects.
   c) Investigate the pathologies of schizophrenia and Alzheimer's disease using PET.
   d) Optimize PET measurements of the receptor occupancy to evaluate therapeutic effects of psychotropic drugs.

2) Molecular Neurobiology
   a) Conduct an exploratory search for therapeutic means capable of pharmacologically alleviating abnormal phenotypes in rodent models of psychiatric disorders, on the basis of mechanistic links between an aberrant monoaminergic neurotransmission and behavioral alterations.
   b) Apply in-vivo imaging systems for Alzheimer model mice to: (i) evaluate novel 18F-labeled amyloid-binding agents potentially useful for early detection and therapeutic assessments of the disease; and (ii) develop imaging probes for differentiation of Alzheimer's disease from non-Alzheimer dementias.
   c) Clarify roles played by peripheral benzodiazepine receptors (PBRs) and allied functional molecules in glial cells toward the therapeutic regulation of neuropsychiatric disorders.

3) System Neurochemistry
   a) Establish experimental environments for transgenic monkeys.
   b) Identify the neural mechanism for addiction and the underlying neurochemical mechanism, especially at the neurotransmitter level.
   c) Carry out trials on a neurophysiological basis for addiction, referring to PET activation results.
   d) Carry out PET analysis of Parkinsonian marmosets.

Progress of Research

1) Clinical Neuroimaging
   b) The inverted U-shaped relation between prefrontal dopamine D2 receptor and the cognitive function (WCST performance) in normal volunteers was found in healthy subjects. With the functional MRI technique, it was revealed that the emotion of “envy” induced neural activation in the anterior cingulate cortex.
   c) PET studies with [11C]DOPA demonstrated that patients with schizophrenia showed an increase in dopamine synthesis rates (k0) in the striatum. A significant correlation between k0 in thalamus and the score of severity of symptoms was also observed. The widespread accumulation of [11C]DAA1106 was observed in the brain of patients with Alzheimer's disease, indicating the expression of PBR due to an activation of microglia.
   d) The measurement of dopamine D2 receptor occupancy using PET was optimized for accurate evaluation of the therapeutic effect of antipsychotics.

2) Molecular Neurobiology
   a) An exhaustive autoradiographic analysis of mice deficient in calcium/calmodulin-dependent protein kinase II revealed prominent changes in levels of multiple monoamine neuroreceptors and transporters by the reduction of this enzyme. In a positron emission tomographic (PET) study of awake rats and monkeys, crosstalk between dopaminergic and glutamatergic neurotransmissions was visualized and quantified in living brains. These results, in conjunction with electrophysiological data using rat brain slices, also elucidated the localization of metabotropic glutamate receptors involved in the regulation of the striatal dopamine release.
   b) Small-animal PET systems showed their utility in characterizing two new 18F radioligands for amyloid plaques developed separately by Tohoku University ([18F]FACT) and a pharmaceutical company. Both tracers were comparable to established 11C-labeled probes in terms of affinities for highly pathological plaque cores, supporting clinical applications of these ligands as diagnostic agents for Alzheimer's disease with advantages over 11C compounds as to the radioactive half-life. We also generated a group of chemicals enabling neuroimaging of fibrillar tau inclusions in Alzheimer's disease as well as related tau-positive neurodegenerative diseases collectively termed tauopathies. Optical and PET scans of tauopathy model mice are being conducted to examine their in-vivo capabilities. These agents in combination with
existing technologies to capture plaque lesions would contribute to separation of Alzheimer's disease from non-Alzheimer tauopathies.

c) Our radiochemical and immunohistochemical assays of animals modeling Alzheimer's disease and other diverse neurological conditions demonstrated that PBR could be upregulated in both microglia and astrocytes, reflecting neurotoxic and neuroprotective roles of reactive gliosis, respectively. This indication was further supported by the finding that levels of PBR and glial cell line-derived neurotrophic factor were correlated with each other in astrocytes responding to neuronal injuries. These insights were obtained with the aid of our original materials, including anti-PBR antibodies and a PET ligand for PBR, [19F]FE-DAA1106.

3) System Neurochemistry

a) In collaboration with another neuroscience facility, we established experimental environments to protect against biohazards derived from monkeys with a specific virus-mediated, specific gene expression at the F2A level.

b) We identified the functional system for psychic dependence on cocaine when monkeys were performing an intravenous cocaine-self administration using PET with 15O-labeled water. Also, we compared dopamine D1 and D2 receptors in extra-striatal regions between pre-addicted and post-addicted monkeys. Unfortunately, we could not find any difference between intra-subject comparisons.

c) We are now studying extracellular unit-recording from the ventral striatum of monkeys that self-administered cocaine. The ventral striatum was chosen for study since our PET activation study indicated it was responsible for the psychic dependence on cocaine.

d) We conducted PET with [14C]PE2I to quantify the extent of dopamine degeneration in MPTP-treated Parkinsonian marmosets. MPTP-treated PD marmosets showed significant decrease of DAT compared with DAT of normal subjects, suggesting the prominent loss of DA terminal.

Major publications


4.3. Studies on Molecular Probes and Radiopharmaceuticals

Toshimitsu Fukumura, Ph.D.
Deputy Director, Molecular Probe Group

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Objectives

Molecular probes play essentially important roles in the rapidly developing molecular imaging field. The purposes of this research group are 1) developing novel probes assessing in vivo biological and physiological functions (Probe Research Team); 2) developing new labeling methods to expand the possibility of producing a wider variety of probes at high yield and high quality (Radiochemistry Team); 3) developing a new integrated system for the production of safe probes considering the GMP standard, without radiation exposure to personnel by automation (Production System Team); and 4) establishing the production methods and the quality control methods of the developed probes for clinical applications (Radiopharmaceutical Production Team).

The Probe Research Team objectives are to develop novel probes for quantitative assessment of oxidative stress and/or disruption of homeostasis and brain efflux function targeting multidrug resistance-associated protein (MRP). This team is also taking part in the development of a novel tumor imaging probe to assess DNA synthesis in tumor cell proliferation and the development of novel receptor ligands. The Radiochemistry Team objectives are to develop new labeling methods with PET radionuclides, especially, a direct fluorination method for 19F onto a benzene ring in an unstable compound and to achieve higher specific activity for various kinds of PET probes. The Production System Team and Radiopharma-ceutical Production Team have not only the above objectives but also have missions to support research activities for PET molecular imaging in collaboration with the Planning and Promotion Unit. The research activities performed in FY 2008 are described below.

Progress of Research

1) Probe Research Team

Our primary role is to develop novel molecular probes for PET and SPECT imaging of molecular targets in humans. The targets are underlying causes of various diseases, such as neurodegenerative disorders and tumors. In particular, we focus on development of probes that allow measurement of oxidative stress or the stress-induced alteration of biological functions, because oxidative stress is considered to be a key feature of the disease process. Such probes would be applicable to investigations of the underlying causes of various diseases and of the mechanisms and efficacies of existing or proposed treatments and therapies.

Multidrug resistance-associated protein 1 (MRP1) protects against toxic compounds and oxidative stress by exporting intracerebral xenobiotics and endogenous metabolites into the blood. The currently available methods for studying brain-to-blood efflux are limited due to either their invasiveness or the ability to provide only a qualitative assessment. To overcoming these limitations, we developed a method for assessing MRP1 function in vivo using PET and a newly developed PET probe, 6-halo-7-[11C]methylypurine. This radioprobe is efficiently converted to its glutathione conjugate (MRP1 substrate) in the brain after intravenous administration. When the molecular probe was administrated to Mrp1 knockout mice, the efflux rate of the radioactivity was reduced to approximately 90% compared with wild-type mice. This is the world’s first method which allows noninvasive and quantitative assessment for exporter function in the living brain.

In close collaboration with the Biophysics Group, Diagnostic Imaging Group and Osaka Prefecture University Biopolymer Chemistry Group, we developed a new thermo-sensitive pegylated liposome which encapsulates doxorubicin (anti-cancer agent), manganese (MRI imaging agent) and technetium-99m (SPECT imaging agent). Encapsulation of technetium-99m and manganese allows the detection of the concentration and decomposition of liposome in a tumor, respectively.

In addition, we developed a non-radioactive reagent for selective measurement of acetylcho-lineesterase (AChE) with Ellman’s method, based on our experience regarding development of radioprobes for measurement of AChE in the brain. The AChE selective substrate for use with Ellman’s method has been desired for the past half century.

2) Radiochemistry Team

The Radiochemistry Team is looking at two subjects: labeling techniques and novel PET ligands. A practical labeling method of [15N]ligands was developed using no-carrier-added [15N]NH, with high specific activity. [15N]Urea and [15N]carbamate were synthesized by reacting precursors (isocyanate, carbamoyl chloride or chloroformate) with [15N]NH2. The precursors were prepared by treating amine and alcohol with triphosgene in situ. These reaction mixtures were not purified and were used directly for [15N]ammonolysis, respectively. Using the one-pot method, [15N]carbamazepin was synthesized for the putative brain imaging.


As a third labeling-related study, a nitroalidol reaction between nitro[11C]methane and formaldehyde was investigated. Controlling all the nitroalidol product, nitroethanol, nitrodiol, and nitrotroil, was accomplished by changing bases, additives, and reaction temperature
in 3 min. 2-Amino[2-13C]ethanol was synthesized as an application of the reaction by treatment with EtONa and EtOH followed by nitro-group reduction.

The team developed [13C]DAC as a novel PET ligand for imaging of PBR in kainic acid-lesioned rat brain. A small-animal PET study determined that [13C]DAC had high uptake in the lesioned region, where PBR density was increased. The high in vivo specific binding of [13C]DAC to PBR is available as a new biomarker for brain injuries, neuroinflammations, and tumors etc.

[13C] Gefitinib was synthesized and used for tumor imaging and evaluation of P-gp/BCRP function. In vivo distribution study on NFSa-bearing mice revealed that [13C]gefitinib specifically accumulated into the tumor. A PET experiment produced a clear tumor image for mice. It was demonstrated that the brain penetration of [13C]gefitinib was related to both P-gp and BCRP. [13C] Gefitinib is thus a promising PET ligand to evaluate the effect of brain penetration of gefitinib by combined therapy with P-gp or BCRP modulators, and to characterize the penetration of gefitinib into brain tumors.

3) Radiopharmaceutical Production Team

Research by this team is intended to establish routine production/quality assurance methods for new PET molecular probes. This includes the development and validation of satisfactory regular production and quality control methods for safe administration into human subjects as well as the evaluation of the toxicity and radiation dosimetry for clinical applications. Four new PET probes ([13C]Ac5216, [18F]TO-002, [13C]gefitinib and [18F]FAZA) were approved by the Institutional Review Board at NIRS and released for clinical research.

Ultra-fast and sensitive high-performance liquid chromatographic methods were established for the quality control of short-lived PET probes. These methods allowed the chemical mass of the PET probes to be determined with ultra high specific radioactivity (>3.7 TBq/μmol) and ultra high-throughput analyses (<1 min) to be carried out for a wide array of pharmaceuticals (>30 probes).

We have developed a method combining on-line multi microdialysis sampling with ultra-high-performance liquid chromatography for the continuous monitoring of radioactive and endogenous metabolites of PET probes. This method allowed highly sensitive radiometric detection with good time resolution and could be successfully applied to continuous and simultaneous monitoring of radioactive and endogenous dopaminergic metabolites in the striatum and cerebellum dialysates of the same rat after the administration of L-[β-13C]DOPA.

A rapid and efficient preparative high-performance liquid chromatographic procedure utilizing a hydrophilic interaction chromatography column and a highly volatile organic mobile phase was established to purify short-lived PET probes. Several 13C-radio probes could be prepared within one half-life of carbon-11 (20.4 min) with sufficient radiochemical and chemical purity and high levels of radioactivity and specific radioactivity.

The chemical impurity tests of [18F]FDG preparations produced in other PET facilities in Japan are being conducted for 213 samples from 108 PET facilities.

4) Production System Team

The Production System Team has been developing new attachments for a versatile synthesis apparatus. A new synthesis unit which supports synthesis of [13C]oseltamivir from a preparation of [13C]acetyl chloride and using the [13C]acetylation reaction and subsequent deprotection reaction, was developed. The synthesis apparatus can produce [13C]oseltamivir in sufficient yield and quality for animal PET studies.

An irradiation system producing 241Am and 85Br has been developed and is being optimized. The system showed high thermal tolerance allowing proton beam irradiations up to 20 μA. Such a high beam current yield for 241Am is near the theoretical thick target yield.

Major publications


reaction and estimation of its excitation function up to 70 MeV. *Nuclear Instruments & Methods in Physics Research Section B.*, 266[5], 709-713, 2008


16) A. Hatori, T. Arai, K. Yamamoto et al.: Biodistribution and Metabolism of Anti-influenza Drug \(^{13}\)C-Oseltamivir and Its Active Metabolite \(^{13}\)C-Ro 64-0802 in Mice. *Nuclear Medicine and Biology.*, 36[1], 47-55, 2009


4.4. Research and Development of the Next-generation Technology for Molecular Imaging

Iwao Kanno, Ph.D.
Director, Biophysics Group

(Outline of Research Career)

Iwao Kanno started his professional career at Akita Research Institute of Brain and Blood Vessels in 1970, where he was an active researcher for 36 years. In 1997, he developed a custom radionuclide emission tomography system using a handmade rotational dentist chair. In 1979 he developed a hybrid type of emission tomography which combined positron emission tomography (PET) and single photon emission computed tomography (SPECT). His efforts were also directed to developing methodology for quantitative assessment of physiological and biochemical parameters from PET and SPECT images. In 1997, he developed a custom radionuclide emission tomography system using a handmade rotational dentist chair. In 2006, he joined NIRS where he continues his research career.

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Objectives

The Biophysics Group works to develop methodologies and technologies for watching, detecting, analyzing and understanding the molecular and physiological signals emitted from humans and other living animals. This is done by using the kinetics of radioactive molecular probes, magnetic resonances signals of protons interacting with molecular probes, multi-photon laser microscopy, and engineering physics for detection and imaging of positron annihilations. The group consists of four research teams. The Imaging Physics Team covers software and engineering physics involved in PET instrument systems. The Biosignal Physiology Team combines molecular information and physiological information measured from MRI and microcirculation facilities on hemodynamic signals relating to neurovascular coupling during neuronal activation. The Data Analysis Team aims to extract quantitative parameters from dynamic PET images taken from human subjects and animals after radioactive ligand administrations. The Magnetic Resonance Molecular Imaging Team develops novel methods and applications for detecting the variable signals from high tesla (7T) MRI and multimodal imaging. These four teams collaborate to assess quantitative molecular mechanisms from in vivo measurements on humans and other animals. The Biophysics Group is thus supporting research and applications of other groups working on molecular diagnostic imaging and molecular neuropsychiatric imaging at the Molecular Imaging Center.

c) Reactive gliosis

Reactive gliosis is an important neuronal response after stroke or spinal cord injury. Recently, it has been a subject of interest in regenerative medicine. We proved that a manganese MRI contrast agent can provide good image contrast for studying reactive gliosis in a rat stroke model and have published our analysis.

d) Immune cell labeling and tracking

Non-invasive in vivo detection of trans-plant cells is an important technique for regenerative biology and medicine. We developed a new nontoxic method for labeling immunocytes that provides MRI signal enhancement. The labeled immunocytes were intramuscularly administered to a rat ischemic leg and heart model and imaged with the 7T MRI.

e) Multimodal therapeutic contrast agent using nitroxy radicals

As a novel nonradioactive methodology, the visible anti-cancer drug “SLENU” was developed for in vivo noninvasive, real-time imaging of blood-brain barrier (BBB) permeability for conventional drugs, using nitroxy radicals as spin labels and MRI.

Progress of Research

1) Magnetic Resonance Molecular Imaging Team

a) Therapeutic drug delivery imaging using temperature-sensitive liposome

We tested doxorubicin-containing liposomal drug delivery imaging in vivo. The multimodal and multifunctional liposome was synthesized as a MRI contrast agent, optical imaging agent and anti-cancer drug with tumor targeting capability. We visualized the drug kinetics, accumulation in the tumor, drug release using a thermo-trigger, and the anti-tumor effect in mouse.

b) Multimodal nano-probe using quantum-dots

Multimodal probes were developed from quantum-dot nanoparticles for both MR and optical imaging. Quantum-dots have higher fluorescence properties than conventional organic dyes. The fluorescence properties were protected by using a hydrophobic structure around the nanoparticle core and MRI contrast agents were facilitated by adding a further amphiphilic silica shell structure. We tested for in vivo applications.

2) Biosignal Physiology Team

a) Diffusion functional MRI

Recently, it has been suggested that diffusion-weighted (DW) fMRI could provide a more direct method of observing neuronal activity. We developed a new MRI sequence where a multiple spin-echo echo-planar-imaging sequence is added after a pulsed gradient spin echo, and we succeeded in extracting the BOLD component from DW fMRI signals. The results suggested to us that the main contribution to heavily diffusion-weighted functional MRI signal is not from the BOLD effect.

b) MR elastography for clinical use

MR elastography (MRE) methods deform a sample using an external vibration system. A transverse driver is widely used, which generates shear waves at the object surface. One of the problems is that shear waves rapidly attenuate at a tissue surface and do not propagate into the body. We compared the shear waves generated by transverse and longitudinal drivers. The longitudinal driver was found to induce shear waves deep inside a porcine liver phantom. These results suggested that the longitudinal driver will allow measurement of the shear modulus deep inside the body.

c) Human studies using evidence-based molecular imaging

We performed collaborative studies with active
clinical sites using evidence-based molecular imaging methods such as MR spectroscopy (MRS), DW imaging, susceptibility imaging, and target-specified enhanced MRI. Proton MRS was applied to pediatric radiology in cooperation with the Kanagawa Children's Medical Center, and 13C MRS was used for diagnosis of liver function with the Institute for Adult Diseases. We succeeded in visualizing tumor structures by diffusion tensor imaging in a collaborative study with the NIRS Hospital. Glycosaminoglycan specific MR contrast enabled us to evaluate dysfunction of cartilages around the knee joints; this was done in a collaborative study with Chiba University and Telko Chiba Medical Center.

d) Direct visualization with fluorescent microscopy

For visualization of cortical vasculature, a bolus of Qdot was intravenously injected and the 3-dimensional vascular structure was visualized with in vivo multi-photon excitation fluorescent microscopy. The vein emerging from the parenchyma was identified by tracking the pial venous networks, and its cross-sectional diameter was measured at the focal point. Three-dimensional vascular images were obtained from the cortical surface to a depth of 0.9 mm with a 0.01-mm z-step. The number density and cross-sectional diameter of veins continuing from the pial networks to the parenchyma were measured at a depth of 0.4 mm.

e) Intracortical microcirculation visualized with multi-photon microscopy

The microcirculatory response to anesthesia in brain tissue was determined with multi-photon excitation fluorescence microscopy. The intracortical capillary dimension and red blood cell (RBC) flow were visualized up to a depth of 0.6 mm from the cortical surface in rats anesthetized with either isoflurane or _chloralose. Significant differences in the capillary diameter and mean RBC speed in single capillaries were observed between isoflurane or _chloralose conditions. The findings indicated that local mechanism for blood flow control may exist at the capillary level to maintain the balance of oxygen supply and demand induced by anesthesia in brain tissue.

3) Image Analysis Team

This team aims to realize algorithms and experimental apparatuses to measure and visualize various functionalities of humans and other animals using PET. For fully quantitative PET molecular imaging, a parametric model analysis based on kinetics of an administered radiopharmaceutical in tissues is conducted. In a practical situation, large noise in the PET data is problematic and, therefore, mathematical image processing techniques should be adopted. We developed and evaluated some new algorithms: omission of arterial blood sampling using an intersectional searching algorithm combined with clustering, a denoising algorithm and partial volume correction using Wavelet transformation, and a bias-free algorithm for neuroreceptor imaging.

Moreover, a quantitative PET scan for mice is important for molecular imaging investigations because of the large variety of genetically modified mice. For the scans, radioactivity in the arterial plasma is required. However, this is difficult to do because of the small size of the mice. The team is investigating surgical methods to insert a small catheter into a mouse artery and a system for arterial sampling. In the arterial blood sampling from mice, the allowed amount of sampled blood is 1 µL and its volume should be measured precisely. We are considering a technique using microfluidic chips to develop a practical µL order blood sampling system. An experimental trial system is being evaluated.

4) Imaging Physics Team

This team proposed an improved OpenPET geometry. The OpenPET geometry is our innovative idea which consists of two detector rings of axial length W each separated by a gap G. The OpenPET mainly has three applications; namely, simultaneous PET/CT, extension of the axial FOV, and in-beam PET, which is known as a method for in situ and non-invasive monitoring of tumor-conforming charged particle therapy. To obtain an axially continuous field of view (FOV) of 2WxG, the maximum limit for G must be W. However, two valleys of sensitivity appear on both sides of the gap. Setting a more limited range for the gap as G=W, which is desirable for filling in the sensitivity valleys, results in not only a shortened gap, but also a shortened axial FOV. Therefore we proposed an alternative method for improving the uniformity of sensitivity by shifting two detector rings axially closer to each other or further apart at the same velocity. We simulated an OpenPET scanner which measures events simultaneously by shifting the detector rings. The results showed that the right and left peaks of sensitivity approach each other upon shifting of the detector rings, and these valleys of sensitivity are effectively recovered.

This team also proposed a new depth-of-interaction (DOI) PET detector design, which was named “Xtal cube”. Recently, small, light, and thin photodetectors such as avalanche photodiodes (APD) or multi-pixel photon counters (MPPCs) have become commercially available as alternatives to photomultiplier tubes (PMTs). In this design, therefore, a number of the small photodetectors are coupled to a 3-dimensional scintillation crystal array at any six surfaces. For a
preliminary experiment to study the characteristics of the new DOI detector, we constructed a crystal block consisting of six layers of a 6 × 6 crystal array with two types of Gd:SiO₂ (GSO) crystals. Each crystal size was 2.9 × 2.9 × 3.75 mm³. To measure how the scintillation photons spread into the whole crystal block and distribute on the surface of the crystal block, the crystal block was coupled to a position sensitive PMT at the bottom surface, where all the crystal block surfaces except for the bottom surface were covered with the reflectors. We irradiated fan-beam gamma rays to the crystal array and studied the scintillation photon distribution by analyzing crystal responses on the 2-dimensional position histogram.

Major publications


3) Z. Zhelev, R. Bakalova, I. Aoki, K.I. Matsumoto, V. Gadjeva, K. Anzai, I. Kanno: Nitroxyl radicals for labeling of conventional therapeutics and noninvasive magnetic resonance imaging of their permeability for blood-brain barrier: Relationship between structure, blood Clearance, and MRI signal dynamic in the brain. Mol. Pharm., 2009


16) A.L. Vazquez, K. Masamoto, S.G. Kim: Dynamics of oxygen delivery and consumption during evoked neural stimulation using a compartment model and CBF and tissue PO(2) measurements.