Progress in Biomedical Imaging

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The year 2000 is an exciting time for biomedical imaging. Major advances are being made in magnetic resonance imaging, ultrasound imaging, optical imaging and nuclear imaging. In all of these areas the development and application of new contrast agents are particularly important. Agents targeting molecular mechanisms are being developed in all of these areas. Specific examples will be given.

Work at Washington University will be discussed in detail. One of the major uses of radiopharmaceuticals is to evaluate various cancer therapies. 16α-F18-fluoro-17β-estradiol (FES) and 2-fluoro-2- deoxyglucose (FDG) were used to assess the efficacy of antiestrogen therapy in patients with breast cancer. The two tracers were administered prior to therapy using tamoxifen. Patients where the glucose metabolism increased over that time period responded to therapy while those with the glucose metabolism was constant or decreased did not respond. The patients who responded also had significantly higher uptake of the estrogen receptor ligand. This type of study could be used to evaluate other types of antiestrogen therapy.

We have developed ligands to study the androgen receptor, these ligands can be utilized to evaluate antiandrogen therapy in prostate cancer.

An index of value to oncologists is a measurement of tumor hypoxia. Fluorine-18 labeled fluoromizonidazole has been developed by the group at Washington University and the University of Washington and shown to be an agent that can assess tissue hypoxia. We have recently labeled diacetyl-bis-(N4-methylthiosemicarbazone), ATSM, with various copper radionuclides and are utilizing copper-60 ATSM to quantify hypoxia. This agent has been validated in several in vitro and animal models and is currently being studied in several human protocols. These protocols involve the application of hypoxia imaging to develop new paradigms for radiation therapy treatment planning. These agents could also be utilized to evaluate which patients should be treated with hypoxic sensitizers.

Copper-64 has been used to label antibodies and peptides and these agents have been shown to be useful for radioamino therapy. Copper-64 ATSM has also been shown to have potential for therapeutical applications. The use of a single agent both for diagnosis and therapy labeled with other nuclides will also be discussed.

Magnetic Resonance for Molecular Imaging

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The capability to incorporate reporter genes into plasmid DNA or directly into the genome of an animal has been the standard tool for monitoring the complex processes of gene regulation and expression. The instruments for reporter gene analysis in small animals using high resolution imaging techniques are now available. SPECT, microPET and high field MRI and optical imaging have, in combination, the sensitivity and resolution to provide images of the processes that control and regulate the expression of genes.

The results from these combined modalities can be used to redefine the complex relationship between gene structure and function. Magentic resonance methods are particularly attractive for molecular imaging due to the wealth of various tissue contrast mechanisms that MR inherently possesses. The active areas of MR research in molecular imaging center on labeled T1- and T2*-shortening contrast agents, using metal chelates attached to a variety of sized particles such as liposomes, proteins, and macro-molecules. These labeled MR-visible molecules can then be labeled with biospecific markers.

Investigators at Stanford (Li, Bernarski, et al) have designed an adhesion receptor integrin avb3 attached to a Gd-labeled liposome to serve as a marker of angiogenic vascular tissue to be a useful diagnostic or therapeutic target for diseases characterized by neovascularization such as cancer. A monoclonal antibody directed to avb3, LM609, has been demonstrated to promote tumor regression by inducing apoptosis of angiogenic blood vessels in human melanomas and breast cancers in SCID mouse/human chimeric models and has also been attached to the Gd-labeled liposome.

In addition, methods involving the use of other nuclei, such as F-19 and O-17 are also being studied for their chemical shift and relaxation properties and may offer future utility. Since MR is one of the least sensitive methods being explored for molecular imaging, experimental animal imaging systems approaching 18Tesla are being used for improved signal and contrast to noise. As we learn more about the power and potential of MR-based molecular imaging, we will find MR being a fundamental modality when used in combination with other more sensitive (PET, SPECT, optical) methods in genetic imaging.

Clinical Applications of Molecular Imaging

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Nuclear Medicine has always been molecular.

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Genetic control of cellular function occurs by influencing the production of proteins, enzymes and receptors by the cell. Since its inception nuclear medicine has been imaging receptors and biologic processes. An early nuclear medicine assay, the radioiodine uptake, is now known to be controlled by Na^+/I^- symporter. This system controls the trapping of radioiodine by both the thyroid and breast (1). The pump is controlled by mRNA, which can be modulated by agents such as retinoic acid. Exposure of MCF-7 cells to retinoic acid stimulated iodine uptake/retention in a dose dependent fashion (2). Now, retinoic acid therapy has been advocated for use in patients with thyroid cancer who have reduced iodine uptake in the tumor. Now we add to our imaging repertoire the ability to specifically identify genetic changes in cells using radiolabeled ligands that localize in receptors coded by reporter genes (3).

The most important clinical role of imaging is influencing the management of disease. A key genetic system built into all cells is that controlling programmed cell death. Apoptosis plays a pivotal role in embryologic development, regulation of the immune system, and as a cause of cell death in viral illness. A lack of appropriate apoptosis plays a role in oncogenesis, where loss of cell death results in excessive cellular proliferation. Excessive apoptosis is seen in the rupture of atheroma and in end stage heart failure. Radiolabeled annexin V localizes on cells undergoing programmed cell death (4). Preliminary clinical studies suggest this agent suggest it will be useful to identify successful therapy in patients with neoplasia, to detect acute stroke and myocardial infarction, and to detect activity in patients with autoimmune disorders such as rheumatoid arthritits and Hashimotos thyroiditis.

In the future, there will be a proliferation of radiopharmaceutical markers like annexin V to define key processes in diseased tissue. The proliferation of these agents assures the molecular future of Nuclear Medicine.

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Brain serotonin synthesis control studied with α-methyl-L-tryptophan imaging

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Labelled α-methyl-L-tryptophan [α-MTrp] has been investigated as a possible tracer for the imaging of regional brain serotonin [5-HT] synthesis. The studies have been performed in laboratory animals using autoradiography (with ^{14}C and ^{3}H-labelled tracer) and positron emission tomography (PET) (using ^{11}C-labelled tracer). Subsequently, the studies have been extended to humans, including both normal subjects as well as patients with various brain disorders (e.g. epilepsy, depression, obsessive compulsive disorder, borderline personality disorder, migraine). Experiments in rats have shown that there is significant co-localization between tryptophan hydroxylase (TPH) and labelled α-MTrp, as well as between 5-HT and labelled α-MTrp. The brain uptake of labelled α-MTrp has been modelled according to a three compartment biological model, and the unidirectional trapping constant has been converted into regional 5-HT synthesis rates by dividing it with the conversion factor and multiplying it with plasma free tryptophan (Trp). Our experiments in rats have shown that drugs known to have an effect on the brain serotonergic system (e.g. fluoxetine, reserpine, D-fenfluramine, Ecstasy, buspirone), without having a significant effect on plasma Trp concentration, affected brain 5-HT synthesis in a non-uniform manner; e.g. 5-HT synthesis decreased in the cell bodies while increasing in the terminal areas. Data suggest that 5-HT synthesis is differently controlled in the terminal areas then its control in the cell bodies. Since TPH is not saturated by either Trp or oxygen, the changes in the blood concentration of these substrates resulted in changes in the brain uptake of α-[^{11}C]MTrp. Experiments in normal subjects suggest that there are brain structures in males in which 5-HT synthesis is higher than that in females, while other structures show higher 5-HT in females than males.

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Measurement of Dopamine Release with [^{11}C]Raclopride and PET

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A relatively new approach in PET neuroreceptor studies provides an estimate of changes in synaptic neurotransmitter concentration. This method determines the change in radiotracer binding levels after administration of pharmacologic agents that affect neurotransmitter levels. For example, amphetamine-induced dopamine release has been extensively studied with receptor-binding radiopharmaceuticals (1, 2). Changes in receptor-binding ligand concentration are determined with either paired bolus studies or the bolus/infusion (B/1) method (3). Both approaches provide a relative index, which is presumably related to the change in neurotransmitter concentration. To develop a more quantitative interpretation of these measures, we previously performed simultaneous PET and microdialysis experiments in rhesus monkeys (2). The [^{11}C]raclopride time-activity data from PET were combined with the dopamine microdialysis data in an extension of the conventional compartment model for analysis of neuroreceptor ligands (4). This model was able to successfully fit the 90-min time-activity data from scans performed at two amphetamine doses with the addition of a single parameter, the KD of dopamine. Using this model, mathematical simulations of B/1 experiments were performed to determine the relationship between the measured change in binding potential and the underlying release of dopamine. The simulations showed that the measured change in binding potential was proportional to the integral of the dopamine pulse.

Although the extended receptor model successfully described the 90-min time-activity data collected in the monkey B/1 studies, further examination of the model suggested some in-consistencies. Because of the rapid clearance of synaptic dopamine after amphetamine administration (halftime of 10-15 min) (4), model extrapolations beyond 90 min predict that binding potential should return towards pre-amphetamine baseline values within 1-2 hours post-amphetamine. However, SPECT studies with the D2 antagonist [^{123}I]IBZM demonstrate long-lived reductions in receptor binding (5). Therefore, we performed a study in rhesus monkey using multiple infusions of [^{11}C]raclopride in a single day to characterize the duration of action of amphetamine

and to test the validity of the extended receptor model (6). Binding potential values were reduced by 30 +/- 3 % following amphetamine, and this reduction remained unchanged for 5-6 hours post-amphetamine. These long-lived changes in binding potential disagreed with the prediction of the extended receptor model and may be due to prolonged alterations in the dopamine system induced by amphetamine (5), including changes in the balance between high and low affinity sites, or changes in free receptor number, e.g., due to internalization. Additional studies are underway to clarify the mechanism of the prolonged amphetamine effect on D2 antagonist binding. These studies use the B/1 design with multiple infusions of hot and cold tracer and 4-point scatchard analysis to assess whether the amphetamine-induced binding potential reduction can be attributed to changes in B_{max}, K_D, or both.

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Dopamine Transporter Imaging in Humans: From Clinical Research to Clinical Practice

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Recent work has underscored the feasibility of imaging dopamine transporters (DAT) in living human brain using high affinity cocaine analogs and positron emission tomography (PET) or single photon emission tomography (SPECT). The in vivo assessment of DAT in healthy subjects has proven to be a useful tool for demonstrating changes in DAT as a function of normal aging and as well as variable expression of DAT for allelic variants of the transporter. In neuropsychiatric patients DAT imaging is a biomarker that provides a powerful means for assessing clinical phenotype and monitoring disease stage. This is most evident in the application of DAT imaging to movement disorder patients. High affinity DAT agents are either available or in development for the diagnosis of Parkinson's disease (PD) with an extensive body of work showing the diagnostic efficacy of these imaging methods. Recent interest and research has extended the evaluation of these radiopharmaceuticals as an objective marker of neuronal loss and disease progression and as a monitoring tool for patients undergoing trials of neuroprotective/neurorestorative treatments. Hence, for the first time, basic questions about the pathophysiology of the disease process in Parkinson's disease can be addressed, including what factors initiate disease progression, what is the duration of disease prior to the manifestation of clinical symptoms, what determines the rate of neuronal loss, and how does the dopamine system change at different stages of Parkinson's disease? Imaging studies to date with 123I-b CIT and SPECT in over 100 PD patients studied serially demonstrate a progressive loss from baseline of approximately 10% per annum, or a rate 10-14 times that of normal reduction found in age-matched healthy controls. This is concordant with 18F-F-dopa PET measures of disease progression. Further, within-patient evaluations over 3-5 years of disease allows the construction of a progression curve with estimate of preclinical pathophysiological processes occurring for 5-7 years prior to the onset of clinical symptoms. This information critically informs the dosing strategy for any putative neuroprotective drug, underscores the desirability for robust means to assess patients at risk for PD, and provides a technique to evaluate the efficacy of disease modifying therapeutics in these disorders.

Development of Receeptor Subtype Specific Radiophamaceuticals based on the Genetic Data and Autopsy Results.

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In vivo imaging with radiotracers is the most sensitive method for studying chemistry at easily saturable sites. Positron emitting radiotracers have been developed to measure these biochemical changes at receptors and enzymes. With the recent sequencing of the genome, the discussion now turns to what role molecular imaging can play in applying this genetic information to alterations in chemistry in the body. These phenotypes, i.e., measurable attributes such as a change in enzyme activity or a change in receptor concentration compared to the norm, are characteristic of the individual and should yield important information in studying the effects of therapy.

The current state of molecular genetics of Alzheimer's Disease (AD) focuses on four genes: the β-amyloid precursor protein (β-APP) gene, the presenilin 1 gene and the presenilin 2 gene. Few of the imaging studies to date have been based on these gene products, although binding to $A\beta_{42}$ protein has been reported. Most of the leads for new receptor binding radiotracers are based on findings at autopsy rather than on genetic defects. On the basis of autopsy data showing changes in the M2 muscarinic receptor, we synthesized 3-(3-(3-[F-18] fluoropropyl) thio)-1,2,5,thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine ([F-18]FP-TZTP). Autoradiography using no carrier added [F-18]FP-TZTP confirmed the uniform distribution of radioactivity characteristic of the M2 pattern of localization in rats. Co-injection of P-TZTP at 5, 50 and 500 nmol inhibited [F-18]FP-TZTP uptake in a dose dependent manner. The difference in brain regions between each dose level was significant.

PET studies in isoflurane-anesthetized rhesus monkeys were performed to assess the *in vivo* behavior of [F-18]FP-TZTP. [F-18]FP-TZTP time-activity curves (TACs) in brain were well-described by a one-compartment model with three parameters: uptake rate constant K_1, total volume of distribution V, and a global brain-to-blood time delay Δt. Tracer uptake in the brain was rapid, with K_1 values of 0.4 to 0.6 ml/min/ml in gray matter. Pre-administering 200 - 400 nmol/kg of non-radioactive FP-TZTP produced a dramatic reduction in total binding of ~50% in cerebellum and 60-70% in other gray matter regions. This reduction was highly significant in all regions. Specific binding values were then determined by the difference between control and pre-blocked V values. In collaboration with NIMH investigators, we recently began studies in normal human volunteers, with the eventual goal of studying patients with Alzheimer's disease. V values, representing total tissue binding, were very similar in cortical regions, basal ganglia, and thalamus, but were significantly higher in amygdala. The methodology and results from our analysis of [F-18]FP-TZTP data in young controls provide the basis for ongoing studies in elderly controls and patients with Alzheimer's disease.

Central Nicotinic Receptor Binding Radiopharmaceuticals

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Central nicotinic acethylcholine receptors (nAchR) are involved in various pharmacological effects of nicotine, such as addiction, seizure, cognition/attention enhancement, analgesia, neuroprotection and in various disease states, such as Alzheimer's disease, Parkinson's disease, and depression. Thus, non-invasive visualization of nAchR in human brain is of major interest in PET and SPECT diagnostic use.

Basic requirements for radiolabeled compound to be useful for in vivo imaging of nAchR include a high and selective affinity to nAchR as well as high brain uptake following peripheral administration. For initial attempts to image nAChR, [^{11}C](-)Nicotine was synthesized due to the high affinity for nAchR and high blood-brain barrier (BBB) penetration. This compound, however, suffers from an elevated degree of nonspecific binding in vivo. Further research efforts, therefore, have focused on the development of highly specific and selective nAchR radioligand able to localize nAchR sites in vivo; some compounds derived from several structural classes, such as (-)nicotine, epibatidine, and 3-pyridyl ether analogs, have been recently developed. In particular, A-85380, a pyridyl ether derivative has shown high and selective affinity for the (x4P2 subtype of neuronal nAChR. However, this compound lacks the readiness for the radiolabelling by simple substitution of an isotopical radionuclide. Therefore, for the development of nAchR imaging agents, we estimated instead, the introduction of heterogeneous group or element as effective and rational radiolabelling site for this nicotine derivative. Nevertheless, the introduction of heterogeneous groups or elements strongly affects the structural, steric and electric properties of the original compound, calling for very restrictive drug design. Structure-activity relationship studies have shown that the binding of nicotine derivative to nAchR may be associated with a cationic pyrrolidine or azetidine nitrogen atom, an electronegative pyridine nitrogen atom, planarity of the pyridine ring, and the distance between the two nitrogen atoms; in addition, any modification of the pyrrolidine or azetidine moiety drastically affects the binding affinity for nAChR. Thus, from the viewpoint of minimum disturbance of receptor binding and maximum in vivo stability, position 5 of the pyridine ring of A-85380 was selected as the most practical site for

[^{11}C]methylatron for PET or [^{123}I]iodination for SPECT radiopharmaceuticals. 5-[^{11}C]Methyl-A-85380 (5MA) and 5-[^{123}I]iodo-A-85380 (5-IA) were prepared from the same precursor, 5-tributylstannyl derivative. Both radiopharmaceuticals showed high and selective binding affinity for nAChRs, high brain uptake and the cerebral regional distribution correlated well with the distribution of nAchR.

These approaches based on structure-activity relationship studies may bring out the development of useful radioligands for receptor imaging.

Plasticity of higher brain function: tactile to visual modalities

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The brain is a dynamically changing structure in relation to learning, alterations of the peripheral body parts or brain injury. Braille reading requires the conversion of simple tactile information into meaningful patterns that have lexical and semantic properties. The primary visual cortex of early blind is known to be activated by Braille reading and other tactile discrimination tasks. To explore the circuitry by which tactual activation of the primary visual cortex in the early blind is elicited, we measured regional cerebral blood flow (rCBF) change with 3 Tesla functional MRI during passive tactile tasks performed by Braille readers blinded early (< 16 y.o. n = 9) and late (> 16 y.o. n = 6) in life, and by 8 sighted subjects as a control group. The tasks included nondiscrimination and discrimination tasks. Tactile discrimination activated the primary visual cortex of the early blind whereas suppressed that of the late blind. Irrespective of the onset of blindness, tactile tasks activated the bilateral association visual cortices of the blind. Tactile stimuli without discrimination activated the dorsal visual cortex whereas discrimination process attributed to the activation of the fusiform cortex. Path analysis with the structural equation model including the posterior parietal and occipital cortices confirmed that in the early blind the discrimination task strengthened the functional connection of the dorsal association visual cortex to V1, and V1 to the fusiform gyrus, compared with those during the nondiscrimination condition. On the other hand, late blind showed decrease in the functional connectivitiy of the dorsal association cortex to V1. These findings suggest that the tactual activation of the primary visual cortex of the early blind may be established by redirecting the tactual information to the association visual cortices, and further redirecting them to the primary visual cortex. The former may be caused by the competitive balance of the input of different modalities in the association cortices where different sensory representations adjoin each other. The rerouting between the primary and association visual cortices is onset-dependent, probably due to higher mode-specificity of the primary visual cortex compared with the association cortices.

THE PHYSIOLOGY OF THE CREATlNE KlNASE REACTION AND REACTANTS IMAGED BY ^{31}P NUCLEAR MAGNETIC RESONANCE

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The creatine kinase (CK) catalyzed reaction, phosphocreatine (PCr)+ADP+H^+ --creatine (Cr)ATP, is closely coupled to ATP metabolism in tissues with high ATP turnover. In brain, the CK system is heterogeneous with cortical gray matter showing higher mitochondrial CK isoenzyme (Ub Mi-CK) and Cr concentrations but lower PCr concentration compared to white matter (1,2). These differences parallel the faster and more variable rates of ATP metabolism in gray matter compared to white matter. These observations are consistent with the proposal that Mi-CK is present predominantly in tissues in which the ATP metabolic rate shows large variations. This association suggests that an important physiological function for the CK system, when the mitochondrial isozyme is present, is coupling ATP metabolism and regulating of ATP concentration in transitions to more rapid rates of ATP turnover. Over the past 1 2 years, studied the physiological role(s) of the CK system and of the UbMi-CK isoenzyme using in vitro metabolic responses to very high ATP turn over in gray matter during seizures. The metabolic responses to seizures include increased cerebral blood flow, glucose and 02 consumptions, and cellular O 2 and oxidation state of cytochrome aa3. Early in seizures cerebral gray matter shows stable ATP, increased PCr, and increased CK catalyzed reaction rate. The PCr increase is seen using l-dimensional chemical shift and multi-echo PCr NMR imaging (3,4). Results of recent studies of mice with deletion of the UbMi-CK gene suggest that these metabolic responses to seizures depend upon the presence of the UbMi-CK isoenzyme (5). Baseline brain CK catalyzed reaction rate constants are higher in mutants than in wild type mice. During chemically induced seizures, the CK rate constant increases only in wild types making rate constants the same in all three groups. These reaction rates are measured in vivo in gray matter plus white matter using the steady state saturation transfer 31PNMR experiment. During seizures the ATP and PCr concentrations decrease in two mutant groups, while in controls the ATP concentration is stable and PCr concentration increases. Acute decreases in the CK catalyzed reaction rate also may be an important mechanism in seizures production (6).

Intraperitoneal injections of guanidinosuccinic acid (GSA) produces seizures within 1 7min. Within this time, the CK catalyzed reaction rate measured in vivo decreases 50-80%. Surprisingly, another epileptogenic guanidine compound, L-nitro arginine methylester, an inhibitor of nitric oxide synthase, also rapidly inhibits the CK reaction rate during the latency period before seizure onset. These studies not only strongly suggest that inhibition of CK by mechanism no ye cause seizures but also that the two reactions catalyzed by CK and nitric oxide synthase are coupled by the guanidine substrates, Cr and arginine. This coupling may be critical in coupling ATP turnover and the local cerebral blood flow increase. An important role of the CK reaction in seizures also is suggested by the observation that Cr supplementation inhibits hypoxia induced seizures in the developing brain (6). In conclusion, the CK system, particularly the

reactant concentrations and the UbMi-CK isoenzyme, is critical in maintaining ATP and, perhaps, functional electrocortical activity in the mature cerebral cortex.

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Novel Implications of Fluorodeoxyglucose Imaging In Dementia

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Molecular neuroimaging has the potential to detect early pathophysiological changes in Alzheimer's disease (AD) in vivo. Examples of molecular imaging in dementia include fluorodeoxyglucose (FDG), amyloid and cholinesterase positron emission tomography (PET). Although cholinesterase imaging will allow unique selection of demented patients who may respond to anti-cholinesterase imaging, recent pathological studies have shown that cholinergic deficits do not occur until relatively late in the AD diseaseprocess. Amyloid imaging is a novel tool that potentially will lead to more accurate in vivo neuropathological diagnosis. However, recent transgenic mice and pathological studies have demonstrated that synaptic dysfunction (most notably glutamatergic) is one of the earliest pathophysiological events in AD and that such dysfunction precedes amyloid deposition. Glucose (as a marker of synaptic function) remains as a molecule to reflect sensitively such earliest changes in AD. Fluorodeoxyglucose (FDG) PET has been extensively used as an aid in the differential diagnosis once a dementing disorder has developed. New applications of FDG PET utilize the technique's capability of detecting very early neocortical dysfunction well before neuropsychological performance becomes abnormal not only in patients with mild cognitive impairment but also in cognitively normal subjects who are genetically at risk for AD. Thus, FDG PET appears to be capable of measuring preclinical abnormalities and physiologic consequences of the process that may be most relevant to the development of dementia.

Similarly, Parkinson's disease patients who later developed dementia demonstrated extensive glucose metabolic abnormalities years before clinical onset of dementia. Dementia with Parkinson's disease often demonstrates cortical Lewy bodies. Despite different genetic background from AD, dementia with Lewy bodies (DLB) shares a glucose metabolic pattern similar to that of AD in the cerebral cortex, but there is also a distinct metabolic reduction in the occipital cortex. We demonstrated a relationship between nigrostriatal dysfunction and occipital hypometabolism, indicating potential pathophysiological importance in subcortical - cortical interactions as a part of pathophysiological features in DLB.

Importance of Hemodynamic Factors in Carotid Artery Occlusion

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The relative importance of hemodynamic factors in the pathogenesis and treatment of stroke in patients with carotid artery occlusion has been controversial. The St. Louis Carotid Occlusion Study was a prospective, blinded, longitudinal cohort study carried out to test the hypothesis that Stage II cerebral hemodynamic failure (increased oxygen extraction measured by positron emission tomography) is an independent risk factor for subsequent stroke in medically treated patients with carotid artery occlusion. One hundred eleven patients (30 never-symptomatic and 81 symptomatic) with carotid occlusion underwent baseline assessment of 17 risk factors together with PET measurements of oxygen extraction fraction (OEF). Every six month telephone contact recorded interval medical treatment and subsequent stroke occurrence during average follow-up of 32 months. Patients, treating physicians and endpoint adjudicator were blinded to PET results.

Of 81 symptomatic patients, stroke occurred in 12/39 with Stage II hemodynamic failure and in 3/42 without [p=.005]. Ipsilateral stroke occurred in 11/39 with Stage II hemodynamic failure and in 2/42 without [p=.004]. Six deaths occurred in each group. After adjustment for 17 baseline patient characteristics and interval medical treatment, the relative risk conferred by Stage II hemodynamic failure was 6.0 (95% CI 1.7 - 21.6) for all stroke and 7.3 (95% CI 1.6 - 33.4) for ipsilateral stroke. Stage II hemodynamic failure defines a subgroup of patients with symptomatic carotid occlusion who are at high risk for subsequent stroke when treated medically.

In 30 never symptomatic patients, ischemic stroke occurred in 1/30 (3.3%). No strokes occurred in the carotid territory to the occluded vessel. Multivariate analysis of baseline risk factors for all 111 patients revealed that age, plasma fibrinogen level and PET findings of high OEF distal to the occluded carotid artery were the only independent predictors of subsequent stroke ($p<.05$). The occurrence of previous ipsilateral symptoms (hemispheric or retinal) was not a significant predictive variable. The lower risk of stroke in never-symptomatic patients was associated with a lower incidence of high OEF (4/30) as opposed to symptomatic patients (39/81, p=.002), but no significant difference in age or fibrinogen level. Never-symptomatic carotid occlusion carries a very low risk of subsequent ischemic stroke. This benign prognosis is associated with a low incidence of cerebral hemodynamic compromise in these patients.

These data support the importance of hemodynamic factors in the pathogenesis of ischemic stroke in patients with carotid occlusion. Increased OEF in symptomatic patients is associated with a high risk of subsequent stroke whereas normal OEF is associated with a low risk. Asymptomatic patients have a low risk of stroke and rarely have increased OEF. Extracranial-intracranial bypass surgery may benefit symptomatic patients with high OEF but a randomized clinical trial will be necessary to demonstrate this. Symptomatic patients with normal OEF and asymptomatic patients have a good prognosis on medical therapy and there is no reason to believe that surgery would be of benefit.

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The creatine kinase (CK) catalyzed reaction, phosphocreatine (PCr)+ADP+H^+ --creatine (Cr)ATP, is closely coupled to ATP metabolism in tissues with high ATP turnover. In brain, the CK system is heterogeneous with cortical gray matter showing higher mitochondrial CK isoenzyme (Ub Mi-CK) and Cr concentrations but lower PCr concentration compared to white matter (1,2). These differences parallel the faster and more variable rates of ATP metabolism in gray matter compared to white matter. These observations are consistent with the proposal that Mi-CK is present predominantly in tissues in which the ATP metabolic rate shows large variations. This association suggests that an important physiological function for the CK system, when the mitochondrial isozyme is present, is coupling ATP metabolism and regulating of ATP concentration in transitions to more rapid rates of ATP turnover. Over the past 1 2 years, studied the physiological role(s) of the CK system and of the UbMi-CK isoenzyme using in vitro metabolic responses to very high ATP turn over in gray matter during seizures. The metabolic responses to seizures include increased cerebral blood flow, glucose and 02 consumptions, and cellular O 2 and oxidation state of cytochrome aa3. Early in seizures cerebral gray matter shows stable ATP, increased PCr, and increased CK catalyzed reaction rate. The PCr increase is seen using l-dimensional chemical shift and multi-echo PCr NMR imaging (3,4). Results of recent studies of mice with deletion of the UbMi-CK gene suggest that these metabolic responses to seizures depend upon the presence of the UbMi-CK isoenzyme (5). Baseline brain CK catalyzed reaction rate constants are higher in mutants than in wild type mice. During chemically induced seizures, the CK rate constant increases only in wild types making rate constants the same in all three groups. These reaction rates are measured in vivo in gray matter plus white matter using the steady state saturation transfer 31PNMR experiment. During seizures the ATP and PCr concentrations decrease in two mutant groups, while in controls the ATP concentration is stable and PCr concentration increases. Acute decreases in the CK catalyzed reaction rate also may be an important mechanism in seizures production (6).

Intraperitoneal injections of guanidinosuccinic acid (GSA) produces seizures within 1 7min. Within this time, the CK catalyzed reaction rate measured in vivo decreases 50-80%. Surprisingly, another epileptogenic guanidine compound, L-nitro arginine methylester, an inhibitor of nitric oxide synthase, also rapidly inhibits the CK reaction rate during the latency period before seizure onset. These studies not only strongly suggest that inhibition of CK by mechanism no ye cause seizures but also that the two reactions catalyzed by CK and nitric oxide synthase are coupled by the guanidine substrates, Cr and arginine. This coupling may be critical in coupling ATP turnover and the local cerebral blood flow increase. An important role of the CK reaction in seizures also is suggested by the observation that Cr supplementation inhibits hypoxia induced seizures in the developing brain (6). In conclusion, the CK system, particularly the

reactant concentrations and the UbMi-CK isoenzyme, is critical in maintaining ATP and, perhaps, functional electrocortical activity in the mature cerebral cortex.

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Novel Implications of Fluorodeoxyglucose Imaging In Dementia

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Molecular neuroimaging has the potential to detect early pathophysiological changes in Alzheimer's disease (AD) in vivo. Examples of molecular imaging in dementia include fluorodeoxyglucose (FDG), amyloid and cholinesterase positron emission tomography (PET). Although cholinesterase imaging will allow unique selection of demented patients who may respond to anti-cholinesterase imaging, recent pathological studies have shown that cholinergic deficits do not occur until relatively late in the AD diseaseprocess. Amyloid imaging is a novel tool that potentially will lead to more accurate in vivo neuropathological diagnosis. However, recent transgenic mice and pathological studies have demonstrated that synaptic dysfunction (most notably glutamatergic) is one of the earliest pathophysiological events in AD and that such dysfunction precedes amyloid deposition. Glucose (as a marker of synaptic function) remains as a molecule to reflect sensitively such earliest changes in AD. Fluorodeoxyglucose (FDG) PET has been extensively used as an aid in the differential diagnosis once a dementing disorder has developed. New applications of FDG PET utilize the technique's capability of detecting very early neocortical dysfunction well before neuropsychological performance becomes abnormal not only in patients with mild cognitive impairment but also in cognitively normal subjects who are genetically at risk for AD. Thus, FDG PET appears to be capable of measuring preclinical abnormalities and physiologic consequences of the process that may be most relevant to the development of dementia.

Similarly, Parkinson's disease patients who later developed dementia demonstrated extensive glucose metabolic abnormalities years before clinical onset of dementia. Dementia with Parkinson's disease often demonstrates cortical Lewy bodies. Despite different genetic background from AD, dementia with Lewy bodies (DLB) shares a glucose metabolic pattern similar to that of AD in the cerebral cortex, but there is also a distinct metabolic reduction in the occipital cortex. We demonstrated a relationship between nigrostriatal dysfunction and occipital hypometabolism, indicating potential pathophysiological importance in subcortical - cortical interactions as a part of pathophysiological features in DLB.

Importance of Hemodynamic Factors in Carotid Artery Occlusion

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The relative importance of hemodynamic factors in the pathogenesis and treatment of stroke in patients with carotid artery occlusion has been controversial. The St. Louis Carotid Occlusion Study was a prospective, blinded, longitudinal cohort study carried out to test the hypothesis that Stage II cerebral hemodynamic failure (increased oxygen extraction measured by positron emission tomography) is an independent risk factor for subsequent stroke in medically treated patients with carotid artery occlusion. One hundred eleven patients (30 never-symptomatic and 81 symptomatic) with carotid occlusion underwent baseline assessment of 17 risk factors together with PET measurements of oxygen extraction fraction (OEF). Every six month telephone contact recorded interval medical treatment and subsequent stroke occurrence during average follow-up of 32 months. Patients, treating physicians and endpoint adjudicator were blinded to PET results.

Of 81 symptomatic patients, stroke occurred in 12/39 with Stage II hemodynamic failure and in 3/42 without [p=.005]. Ipsilateral stroke occurred in 11/39 with Stage II hemodynamic failure and in 2/42 without [p=.004]. Six deaths occurred in each group. After adjustment for 17 baseline patient characteristics and interval medical treatment, the relative risk conferred by Stage II hemodynamic failure was 6.0 (95% CI 1.7 - 21.6) for all stroke and 7.3 (95% CI 1.6 - 33.4) for ipsilateral stroke. Stage II hemodynamic failure defines a subgroup of patients with symptomatic carotid occlusion who are at high risk for subsequent stroke when treated medically.

In 30 never symptomatic patients, ischemic stroke occurred in 1/30 (3.3%). No strokes occurred in the carotid territory to the occluded vessel. Multivariate analysis of baseline risk factors for all 111 patients revealed that age, plasma fibrinogen level and PET findings of high OEF distal to the occluded carotid artery were the only independent predictors of subsequent stroke ($p<.05$). The occurrence of previous ipsilateral symptoms (hemispheric or retinal) was not a significant predictive variable. The lower risk of stroke in never-symptomatic patients was associated with a lower incidence of high OEF (4/30) as opposed to symptomatic patients (39/81, p=.002), but no significant difference in age or fibrinogen level. Never-symptomatic carotid occlusion carries a very low risk of subsequent ischemic stroke. This benign prognosis is associated with a low incidence of cerebral hemodynamic compromise in these patients.

These data support the importance of hemodynamic factors in the pathogenesis of ischemic stroke in patients with carotid occlusion. Increased OEF in symptomatic patients is associated with a high risk of subsequent stroke whereas normal OEF is associated with a low risk. Asymptomatic patients have a low risk of stroke and rarely have increased OEF. Extracranial-intracranial bypass surgery may benefit symptomatic patients with high OEF but a randomized clinical trial will be necessary to demonstrate this. Symptomatic patients with normal OEF and asymptomatic patients have a good prognosis on medical therapy and there is no reason to believe that surgery would be of benefit.

Neural and molecular mechanisms of fatigue sensation

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Fatigue is sensed in the brain, and some molecular and neuronal mechanisms underlie in the sensation. However, our knowledge about the brain mechanisms of sensing fatigue is limited although this might be a typical problem of neuro-immune-endocrine interaction. One of the interesting pathologies in the fatigue research is chronic fatigue syndrome (CFS) whose cliteria have been defined as prolonged generalized fatigue, muscle weakness, myalgia, postexertional malaise, fever with unknown etiology, and lymph node pain. Many hypotheses were proposed for the etiology of this syndrome, including viral infection. Some candid viruses were isolated, but the entire mechanisms which explain the relation to fatigue sense are not clarified yet. Recently, Kuratsune et al. (1) found the serum acylcarnitine (ACR) deficiency in the vast majority of patients with CFS. Serum ACRs are the bound form of short chain fatty acids to carnitine, mostly acetyl-carnitine, but their physiological roles are still unclear. To clarify this, we investigated the dynamics of acetyl-L-carnitine labelled with ^{11}C in different positions by use of PET (2-4), and found that mammalian liver can immediately salvage and store unused ACR when the supply of energy source is sufficient, and that a large amount of [2-^{11}C]acetyl moiety was taken up into the brain and converted to glutamate. The lower uptake of [2-^{11}C]acetyl-carnitine was observed in Brodmann's areas 9/46d and 24 in CFS patients as compared with age-matched normal controls (n=8 in each). There is no specific correlation between the sites which show lower ACR uptake and those lower rCBF in CFS patients. The study on the fatigue neural circuit is currently in progress in our laboratory using animal model.

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PET Studies of Acute Pathophysiology in the Neurointensive Care Unit

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In September 1995, a PET scanner was installed in the Barnes Hospital Neurointensive Care Unit at Washington University Medical Center. During the past 5 years we have used this scanner to investigate the pathophysiology of acute neurological disease.

The effect of pharmacological blood pressure reduction on the regional CBF around the clot in patients with acute intracerebral hemorrhage (ICH) has been investigated in 12 hypertensive patients with supratentorial ICH within 24 hours after onset. They underwent measurements of CBF and then received nicardipine (n=5) or labetalol (n=7) to produce a 15% reduction in mean arterial pressure (MAP). CBF measurements were then repeated. All PET images were masked to exclude non-cerebral structures and corrected for partial volume effects due to clot, CSF spaces and skull. MAP was reduced from 142 ± 9 to 120 ± 8 mm Hg. Global CBF was 40 ±12 at baseline and 37 ± 8 ml $100g^{-1}$ min^{-1} after MAP reduction (95% CI of the difference = +2 to -6). CBF in a 1 cm region around the clot was 21 ± 8 at baseline and 21 ± 8 ml $100g^{-1}$ min^{-1} after blood pressure reduction (95% CI of the difference = +0 to –2. MAP reduction of this degree does not lead to a reduction in CBF in the area surrounding acute intracerebral hemorrhage.

In a study of 16 patients with acute intracerebral hemorrhage, we compared CBF, OEF and $CMRO_2$ in the region around the clot to a mirror region on the contralateral side to determine if there was local ischemia produced by the clot. In the peri-clot region CBF and $CMRO_2$ were reduced (CBF: 21 ± 8 ml $100g^{-1}$ min^{-1} vs contralateral 37 ± 14 ml $100g^{-1}$ min^{-1}, $p < .001$; $CMRO_2$: 1.4 ± 0.5 ml $100g^{-1}$ min^{-1} vs contralateral 2.9 ± 1.0 ml $100g^{-1}$ min^{-1}, $p< .0001$). However, OEF was reduced in the peri-clot area as compared to the opposite side (0.44 ± 0.18 vs contralateral 0.51 ± 0.13, p=. 05) not elevated, indicating that there was no ischemia in the peri-clot region when these scans were performed a median of 16(range 5-22) hours after onset.

We have also investigated whether the reduction in CBF produced by hyperventilation (HV) in patients with traumatic brain injury is severe enough to reduce cerebral oxygen supply below that needed to meet metabolic needs. We measured CBF, OEF and $CMRO_2$ with PET before ($PaCO_2$=38±4 mm Hg) and during HV ($PaCO_2$=29±1 mm Hg) in 8 subjects with severe TBI (Glasgow Coma Score 3-7) 13±6 hours after injury. Baseline hemispheric CBF of 38±14 ml $100g^{-1}$ min^{-1} decreased to 26 ±9 ml $100g^{-1}$ min^{-1}during hyperventilation ($p< .001$). To study regional effects of HV, an automated search routine was used to identify 5 ml spherical regions with the lowest CBF during each patient's HV study. Regional $CMRO_2$ before and during HV were compared for these regions. There were no significant changes in $CMRO_2$ for those regions with CBF <20 during HV (before 1.2±.3 vs during 1.2±3, p= .86), CBF <15 during HV (1.0±.2 vs 1.0±.3, p=.75) or CBF <10 during HV (0.8±3 vs 0.9±4, p=.70). We concluded that moderate HV to $PaCO_2$ 29±1 mm Hg in patients with severe TBI did not produce regional deterioration in $CMRO_2$ in areas where CBF dropped to the lowest levels. This was due to low pre-HV $CMRO_2$ in these areas and adequate compensatory increases in OEF during HV. We, therefore, found no evidence of regional ischemia under these conditions.

Stroke Recovery and Motor Reorganization

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One of the main advantages of functional magnetic resonance imaging (fMRI), especially fMRI performed on high field systems, is the fact that sophisticated activation studies can be readily performed on an individual case basis within a single session. This fact is especially important in the clinical setting where it is virtually impossible to perform multi-subjects-single image statistics standard in positron emission tomography (PET). Appropriately chosen dependent variables defined by individual activation studies allow for high quality statistical analysis utilizing multiple patients who inherently exhibit substantial variation in their activation.

Independent component-cross correlation-sequential epoch (ICS) analysis of activation of the primary motor cortex unequivocally demonstrated that the primary motor cortex of the hand possesses two discrete areas, namely, area 4 anterior and area 4 posterior. ICS analysis in patients with hemiplegia due to internal capsule infarction and patients with monoplegia due to brachial plexus injury indicated that primary motor area 4 posterior (MI-4p) represented the "initiation" cortex for voluntary hand motion.

Further studies in stroke patients disclosed that patients who exhibited substantial functional recovery (stage 5-6 of Brunnstrom's classification) within 30 days did not show significant motor reorganization. In contrast, patients who did not exhibit substantial functional recovery within 30 days showed significantly higher rates of motor reorganization characterized by activation of the ipsilateral primary sensoriomotor cortex (SMl) and corresponding supplemental motor area (SMA). Furthermore, activation of ipsilateral SMI and SMA were significantly frequently observed in patients who eventually had substantial functional recovery (stage 5-6) within 90 days.

The presence of MI-4a activation provides a reliable index of the integrity of the central system of voluntary motor function. Early recovery of hemiplegia in subcortical stroke patients likely represents recovery of the "primary" motor system contralateral to the affected hand. In contrast, late recovery represents development of bi-hemispheric control. Cerebellar re-organization appears to be a consequence of hemispheric reorganization.

Tumor Imaging Using Metabolic Tracers

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Background

The purpose of tumor imaging with metabolic tracers is not only to detect tumors but also to understand biological properties of tumors. Biological properties should be easily translatable into general medical terms such as malignancy. For this purpose, theoretical background is essential. A 2-deoxyglucose analog, [^{18}F]FDG, is the most frequently used tracer to visualize tumors in clinical situation. [^{18}F]FDG has high sensitivity to diagnose several types of tumors (1). This sensitivity is based on the high rate of glucose metabolism in tumors. Many trials have shown that [^{18}F]FDG is useful in the diagnosis/staging of malignancy. On the other hand, the theoretical relationship between the amount of FDG retention and malignancy is not yet clear nor the theoretical reason for the differences in glucose metabolic activity in various tumors (2). We present here the results of our systematic *in vitro* investigation to study FDG/DG retention mechanisms and their differences among tumor cell lines. In addition, we are currently developing a new approach to extract information on mitochondrial function of tumor cells using [^{11}C]acetate. Therefore the character of [^{11}C]acetate as a tumor imaging agent will be also discussed.

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Thymidine Analogs for Imaging DNA Synthetic Pathways

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One of the most important properties of tumors is their rapid proliferation. Agents to image this aspect of tumor metabolism have been widely sought in order to detect cancers and measure their response to therapy. To this end a number of potential tracers have been explored over the years and these have included [C-11]thymidine, [F-18]FLT (3'-deoxy-3'-fluorothymidine), FMAU (2'-fluoro-5-methyl-1-beta-D-arabinofuranosyluracil), and IUdR (5-iodo-2'-deoxyuridine). Investigations have been performed to image tumors with [C-11]thymidine and it has been shown to be readily retained in a number of tumors and measures the response to therapy in pilot studies (I). Because of the biodegradation of thymidine, to quantitively measure DNA synthesis, one needs to analyze its metabolites, as well as the activity retained in DNA.

The difficult synthesis of [C-11]thymidine and its rapid catabolism limit its use in routine clinical settings. Investigators have, therefore, sought other nucleoside analogs that can resist catabolism, can be trapped in the DNA synthetic pathway, and would be more easily labeled -optimally with F-18. FLT is one anti-viral compound that has been put to this use. We did initial studies of FLT in mice and dogs. In proliferating tissues of mice FLT retention was variable, probably the result of high blood levels of free thymidine in rodents. Studies in dogs demonstrated high retention in marrow and tumors. FLT can be readily labeled with F-18 and in vitro incubation in blood has shown that it is resistant to the initial catabolic enzyme thymidine phosphorylase (2). In animals it was largely excreted unchanged in the urine. [F-18]FLT is retained in tumors and proliferating tissues after phosphorylation by thymidine kinase (3). Its uptake provided a relative measure of proliferation, similar to the measurements of glucose utilization provided by FDG. Within the thorax FLT can be readily used to image tumors, since background uptake is very low, aside from the normal uptake in the proliferating bone marrow. It can also be used to image high grade brain tumors, since FLT does not readily cross the normal blood-brain barrier. In the abdomen it can be used outside of the liver and urinary system where the physiologic presence of FLT is evident. While studies in a number of species have shown that FLT resists degradation, in humans FLT is glucuronidated and this leads to temporary retention in the liver, FMAU can be labeled with C-11 or F-18 for imaging. It is retained in tumors and proliferating tissues after phosphorylation by thymidine kinase and subsequent incorporation into DNA. Studies in vitro and in animals have shown that it undergoes little degradation. Further work is needed to determine its relative utility as a practical clinical tracer.

Further work is ongoing to determine the relative merits of proliferative tracers and FDG. Depending on the type of tumor under study, its location, and the type of

treatment being employed, one may find certain advantages with one tracer or another. The ease of use of some of the new tracers should lead to greater study and commercial availability.

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Noninvasive imaging of *HSV1-tk* marker gene with FIAU for monitoring transfer and expression of other therapeutic genes by multi-gene delivery vectors.

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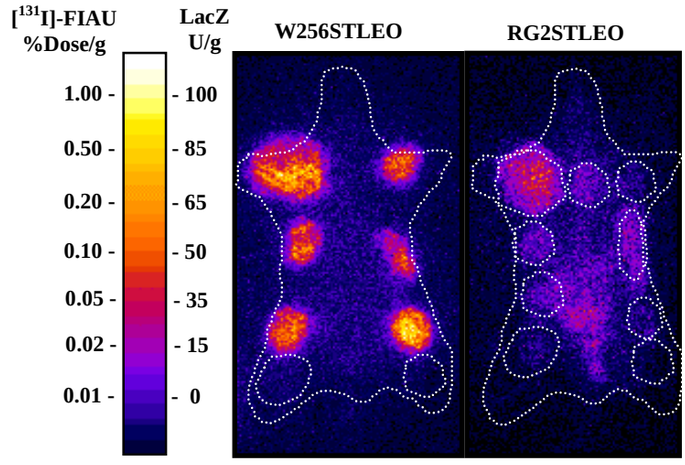
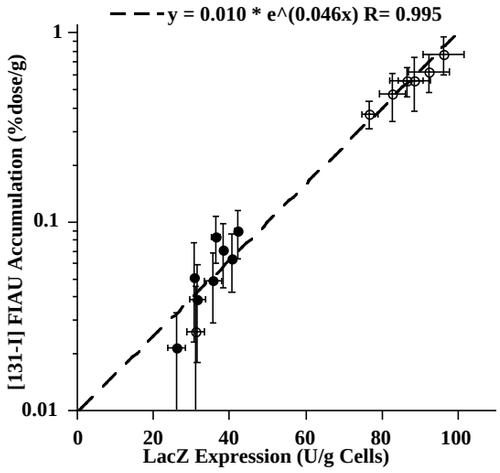
Objectives. The aim of this study was to develop a method for monitoring the efficacy of therapeutic gene transfer and expression by measuring the expression of a marker gene using conventional clinical imaging techniques. An *in situ* marker and a selection gene, the E. Coli *Lac.Z.* and *NeoR* fusion gene (*Lac.Z./NeoR*), were used in a model vector to study the delivery and expression of other therapeutic genes. The *HSV1-tk* and *Lac.Z./NeoR* fusion genes were placed in a bi-cistronic IRES-based expression cassette of a retroviral vector termed STLEO. Studies were performed with this model vector construct to demonstrate stable and proportional co-expression of both genes. The *HSV1-tk* gene was used as a marker gene for *in vivo* imaging.
Methods. *In Vitro*. The RG2 and W256 tumor cells were transduced *in vitro* with the STLEO vector. Several single cell-derived transduced clones were obtained by G418 selection. The level of *Lac.Z.* expression in different clones was measured by quantitative spectrophotometric assay with O-nitrophenyl-galactopyranoside (ONPG) chromagen and expressed as U/g cells. The level of *HSV1-tk* marker gene expression in the same clones was assessed by measuring 2'-fluoro-1-•-D-arabinofuranosyl-5-iodo-uracil [^{14}C]FIAU) accumulation. *In Vivo.* Multiple s.c. tumors were produced from the transduced RG2STLEO and W256STLEO clonal cells in SD (n=12) and RNU/rnu rats (n=4), respectively. The wild type W256 and RG2 s.c. tumors served as control. Three weeks later, animals were injected i.v. with 200 µCi of [^{131}I]FIAU and gamma camera imaging was performed after 24 hours to assess *HSV1-tk* gene expression ([^{131}I]FIAU %dose/g). Tumors were dissected and radioactivity was assessed by gamma spectroscopy. *Lac.Z.* gene expression was assessed in tumor tissue sample homogenates by the spectrophotometric assay (LacZ U/g).
Results. A significant correlation (r>0.9) was observed between corresponding levels of *Lac.Z.* and *HSV1-tk* gene expression in different W256STLEO and RG2STLEO clones in vitro. In s.c. W256STLEO and RG2STLEO tumors, the level of *HSV1-tk* gene expression ([^{131}I]FIAU %dose/g) correlated (r>0.9) with that of the *Lac.Z.* gene expression (U/g) over a wide range of co-expression levels. FIAU has been labeled with [^{124}I] and quantitative PET imaging





has been performed by us previously.

Conclusions. The observed correlation between the levels of *Lac.Z.* and *HSV1-tk* gene expression, both *in vitro* and *in vivo*, demonstrates the potential for monitoring therapeutic gene transfer and expression by non-invasive imaging of the *HSV1-tk* marker gene with [^{123}I]- and [^{124}I]-FIAU. Gene therapy vectors that include the IRES sequence for bi-cistronic co-expression of therapeutic and marker genes are the most likely candidates for successful monitoring of gene therapy in patients in the future.

Imaging and Therapeutic Applications of Bromine and Iodine Isotopes

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We are currently optimizing a system for the rapid distillation of bromine and iodine isotopes of interest in nuclear medicine. The radio-isotopes, ^{76}Br, ^{77}Br and ^{124}I, are produced by the (p,n) reaction on enriched $Cu_2{}^{(76\text{ or }77)}$Se or $Cu_2{}^{124}$Te targets using 14.5 MeV protons. The Br production is based on published work (1). In our approach, the target material is melted in a depression on a platinum coated tungsten disk. The disks are then bombarded windowless and are cooled from the back with a jet of chilled water using the same target holder that has been described for our ^{64}Cu production system (2).

Production rates are as predicted and beam intensities of up to 5μA can be utilized without melting or lost of target material. The separation is performed by thermal distillation in an induction furnace under a flow of argon. We are interested in the induction furnace approach because it allows for a shorter distillation time and a more compact design than that offered by conventional furnaces. The radioactivity is deposited directly on the quartz tube, this is recovered by rinsing with a few mL of NaOH (0.01N), which is then evaporated to dryness and utilized in radiolabeling experiments. The targets can be irradiated a number of times before having to be replenished.

At present our research with these isotopes is broadly divided into two areas, imaging and therapy. All of the isotopes, but especially bromine-77, may have potential as therapeutic agents. In 1982 [^{77}Br]bromodeoxyuridine (BrUdR) was demonstrated to be effective in killing V79 cells (3). We are basing our therapeutic research on this observation are currently evaluating the cell killing effects (in MCF-7 cells) of the two bromine isotopes using BrUdR, prepared by electrophillic substitution of the trimethyltin precursor. (4) We plan to extend this research to compare the effects of delivering the radioisotope directly to the DNA, via deoxyuridines, with the cell killing effects of radiolabeled steroid ligands. Our target steroids are estrogens, 16α-bromo-11ß-methoxyestradiol-17ß (5); and androgens, 7α-halo-5α-dihydro-testosterone.

Progress in both the imaging characteristics of these isotopes on the microPET and their therapeutic applications will be reported.

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