Nano-bio-imaging with Radiopharmaceuticals and its Application to Health Sciences

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1. Introduction

1.1. Nano-bio-imaging

"Nano-bio-imaging" is the entirety of various kinds of imaging techniques based on "nanotechnology". It enables further progress of research in the fields of nanomedicine and nanotechnology. In nuclear medicine technique, one of the very useful techniques for nano-bio-imaging, we administer a very small amount of radiopharmaceuticals to the body of human subjects to investigate "endo-phenotypic alterations" taking place in a living body from the outside. The foundation of this technique could date back to the early 20th century when it was originally developed as a "tracer" technique by Dr. George von Hevesy, a Nobel laureate in chemistry in 1943. This method has continued to make progress, and now it has come to be applied to human studies and then has been started more often to be called "nuclear medicine" in the latter half of the 20th century. The initial system was called scintigraphy, and later progressive innovation has been continued. The technique was later fused with the technology of computed tomography, and has been established as the "single photon emission computed tomography (SPECT)" and "positron emission tomography (PET)" in the late 20th century, both of which have been most suitable tools for nano-bio-imaging. One of the most important advantages of these techniques have been the fact that they can visualize pharmacodynamic/pharmacokinetic information in the tissue of living subjects by injecting only a tiny amount of radiopharmaceuticals (one nano-mole or so). Here, the term "tracers" mean radiopharmaceuticals to be injected in purpose of obtaining signals regarding phenomena in the living body, and sometimes can also be termed "a probe", meaning that we can probe the presence of extremely-small amounts of biological substances. Thus, in the procedure of nuclear medicine, radiolabelling technique with high specific radioactivity is very important to visualize the presence of small amounts of bio-active substances at a "nano-" to "pico-" mole level. Therefore, this technique is the right tool optimized for nano-bio-imaging. Here, the term "a tracer" means injected radiopharmaceuticals used for obtaining information (signals) on phenomena in the body, and sometimes can also be called "a probe", meaning that we can probe the presence of extremely small amounts of biological substances. Thus, in the nuclear medicine technique, radiolabelling with high specific activity is very important to visualize the presence of very small amounts of materials in a "nano-" to "pico-" molar order. Therefore, it can be said that this technique is the right tool optimized for nano-bio-imaging.

Our group has been seeking further potential and new applications of this radionuclide technique to clinical evaluation/diagnosis and health promotion science. We are aiming at promoting the medical use of the radionuclide technique and its further applications to health promotion science; mainly in terms of image analysis and medical engineering research and development as follows:

Project 1) Application of nano-bio-imaging technology to health promotion science and preventive medicine,

Project 2) Development of a new positron diagnostic system, positron emission mammography (PEM), and related software for image processing, and

Project 3) Modeling in nano-bio-molecular imaging including neural transmission and amyloid imaging.

In 2008, we have mainly promoted the following study themes, particularly on PET studies.

1.2. Information available from the living human brain

Human mental function, of course, is based on the actions of our brain network. On one hand, one ultimate and fundamental aim of this study topic is "elucidation of psychiatric functions of human brain". On the other hand, in the modern society, incidence of neuropsychiatric disorders such as depression, anxiety disorder and cognitive disorder (dementia) is constantly increasing. A large amount of "stress" in our daily lives has been associated with the increased rate of various psychiatric disorders. When we think about a structured strategy to overcome such problems, we find that the imaging technology could be able to serve basic data for building up a practical strategy.

As for the methodology for nano-bio-imaging, we can use PET to measure cerebral energy (glucose) metabolism by injecting a small amount of radio-labeled glucose ([^{18}F]fluorodeoxyglucose: [^{18}F]FDG). Due to the activated regional brain metabolism, the demand for glucose and oxygen increases, inducing dilation of cerebral capillaries, which is observed as an increase in the regional cerebral perfusion. Cerebral perfusion can be measured using radio-labeled water ([^{15}O]H_2O: Fig. 1). More recently, a method using magnetic resonance imaging has also been applied to the measurement of brain regional perfusion (functional MRI: fMRI). In addition, another technique using near-infrared light (NIRS) has been introduced for measuring brain regional perfusion. However, PET is still in use very often, mainly for measuring regional brain glucose consumption and for evaluating neuro-transmission function (Fig. 1). In the human brain, neurotransmitters can manifest their effects even in very small amounts. It is not easy to visualize the actions of neurotransmitters in the living human brain externally without using a highly sensitive technique such as PET (Fig. 1).

Presently, the regional cerebral blood flow can be measured using various methods such as PET, fMRI and NIRS. Interactions of neurotransmitters and receptors can be evaluated mainly using PET. In the human brain, neurotransmitters can manifest their potent effects even with very small amounts. It is not easy to visualize the actions of neurotransmitters in the living human brain externally without using a highly sensitive technique such as PET [1]. Using PET, it is possible to quantify interactions between neurotransmitters and neuroreceptors as well as tissue metabolism in the living brain. Our group has studied neurotransmission of the histaminergic and dopaminergic neuronal systems, by constructing a model and a simplified quantification method that can be applied to further clinical studies [2]. (Fig. 1)

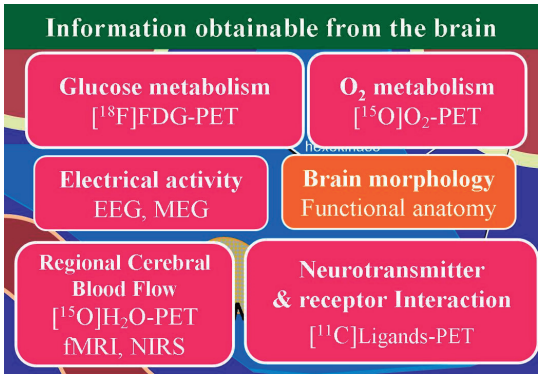

Fig. 1. Information available from the living human brain. The most important energy resource of the human brain is glucose. Oxygen is necessary for glucose metabolism. These substances are supplied by the blood stream. Brain regions with increased activity are accompanied by increased regional cerebral blood flow. Information regarding glucose and oxygen metabolism can be obtained using PET.

2. Application of Nano-bio-imaging Technique to Health Promotion Science and Preventive Medicine

2.1. The definition of health

According to the definition by the World Health Organization (WHO), "health" is "a complete state of physical, mental and social well-being, and not merely the absence of disease or infirmity." Needless to say, quality of life (QOL) is very important in all procedures of medical treatment and care. The definition may suggest that attention should also be paid to QOL in daily lives. In general, there appears to be greater anxiety about the issue of radioactivity in Japan than in Western countries. A highly-sensitive PET scanner has enabled examinations at minimal radiological doses, far below the annual environmental exposure of 2.4 mSv. This has allowed PET to be applied to elucidate the mechanism of our physical functions that achieve and maintain a healthy state. This could be viewed as a new application of PET to the field of preventive medicine and health promotion.

Our studies of the brain activities of patients who suffer from malignant diseases have yielded interesting results. The brain metabolic pattern of cancer patients show abnormalities that correlate to the intensity of depressive mood. Also psychological state correlates to immune functions possibly via the regional brain functions. PET appears to be useful not only for diagnosis of cancer but also for evaluation of the psychological status of cancer patients. Furthermore, we have conducted studies regarding whole-body exercise such as running and bicycle riding to observe regional activities of skeletal muscles, heart muscles, and brain. PET was useful for evaluating the effects of various therapeutic interventions including exercise for metabolic disorders such as diabetes mellitus, hypertension, cerebro- and cardio-vascular diseases in terms of interaction between the mind, brain and body. Recently, alternative medicine has become popular among patients, and the scientific evaluation of these therapeutic techniques would be of clinical importance. PET can be very useful for evaluations based on scientific evidence.

We have planed to investigate the relationship between psycho-behavioural factors and achieving a healthy state. Our ultimate goal is to establish a model of whole-body organ interactions.

2.2. Exercise and brain imaging study related to "physical and mental health"

In the scope of applying the PET method to health promotion science, we have performed imaging studies during various forms of exercise, including running and bicycle riding, scanning not only their brain but also cardiac and skeletal muscles [3-6]. By conducting the whole-body scanning, we can obtain a whole-body map of energy metabolism in the human body. To date, there are few study groups using PET for health

promotion science, and this topic will have great relevance in the future. We are currently collaborating with Professor Toshihiko Fujimoto at the Center for the Advancement of Higher Education / Department of Medicine and Science in Sports and Exercise in this new area of research.

We have analyzed regional brain activities with an emphasis on the brain's special role as the important center controlling the interaction between muscle action and immune functions. Our series of exercise studies have attracted the attention of overseas researchers. As an example, Professor Arne Dietrich (Department of Social and Behavioral Psychology, American University of Beirut: AUB) has shown his interest in our research. His attention has been focused on the importance of a brain energy metabolism distribution mechanism during exercise, and he established the "transient hypofrontality theory" (THT) [7,8]. As a method to investigate his theory, we have started new collaborative work to determine directly using PET with [^{18}F]FDG (exercise PET) to examine the importance of measuring energy consumption during ongoing exercise, running a joint-protocol with CYRIC, Tohoku University and AUB.

Then, we first applied the [^{18}F]FDG PET to human subjects during a running task in the upright posture, and demonstrated augmented energy consumption in the parieto-occipital region during the task compared with the motor area [6]. This was probably due to the higher energy consumption necessary for integrating multimodal sensory information. Our results also showed that frontal activity was reduced during running than during resting. In addition, our study demonstrated a trend of relative decrease in whole brain mean activity during exercise compared with the resting condition. In parallel, our group also examined whole-body energy (glucose) redistribution and how exercise affects this distribution. We showed no significant changes in relative glucose metabolism between exercise and resting conditions [4]. Based on our work, Kemppainen and coworkers examined absolute glucose consumption in the human brain of healthy volunteers. They confirmed, for the first time, using [^{18}F]FDG PET technique, that the glucose consumption level decreases during strenuous exercise especially in the cingulate gyrus [9] (Fig. 2).

We demonstrated the relative increase in glucose uptake in the temporo-parietal association cortex, occipital cortex, premotor cortex, primary sensorimotor cortex and the cerebellar vermis [6]. Relative reduction in glucose uptake was detected in the prefrontal cortex, temporal cortex, cerebellar hemisphere, brain stem, striatum. Mean values of global brain glucose uptake was relatively lower in runners than in resting controls [6]. Kemppainen and coworkers later demonstrated significant reduction of regional glucose metabolic rate in all cortical regions in correlation to exercise intensity [9]. They also pointed out that exercise could be associated with adaptive metabolic changes in the frontal cortex [9]. Thus, global and regional brain metabolic decline was observed using [^{18}F]FDG PET especially in the limbic and frontal regions [6,9,10]. It is easy to explain the metabolic increase in the regions directly associated with execution of exercise task, while it is not so easy to explain the mechanism of relative decrease in the regions not involved in exercise. Previous imaging studies in anxiety disorders demonstrated increased glucose metabolism in these regions [10-12]. We speculated that the metabolic reduction in the frontal and limbic regions was associated with emotional changes in runners, including the phenomenon called "runner's high" [13] (Fig. 2).

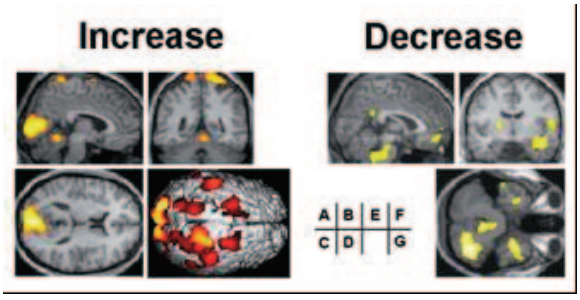

Fig. 2. Relative glucose metabolic differences between controls (resting) and running subjects demonstrated in terms of adjusted regional glucose metabolic rate ratios. Brain regions of statistically significant difference are shown in this figure. In all areas, $p < 0.001$, compared controls (resting) and running subjects. Symbols: ** = Z score > 4.0, * = Z > 3.0. Modified from the reference [6] by courtesy of Minerva Medica.

Regarding the association with psychological factors and the regional brain functions, Dietrich and Sparling have reported that endurance exercise impaired prefrontal-dependent cognitive ability in healthy volunteers [8]. Dietrich later proposed a new theory (transient hypofrontality theory: THT) to explain the metabolic reduction in the prefrontal region [7], where the prefrontal activity is suppressed indirectly due to the limitation in energy supply to the brain [7]. Interestingly, this theory also explains a neural mechanism regarding the mental health benefits of exercise [7,8]. Here, it is of interest to also point out that Kemppainen and coworkers have suggested that substrates other than glucose, most likely lactate, are used by the brain as energy source in order to compensate the increased energy demand to maintain neuronal activity during high intensity exercise, since lactate availability during exercise tended to correlate negatively with the brain glucose uptake measured with [^{18}F]FDG PET in their study [9].

In addition, we have been studying the interrelationship between the regional brain activity and endocrine and immune systems during whole-body exercise. The specific aim of this study was to examine

the relationship between natural killer cell activity (NKA) and the regional brain activity under influence of whole-body exercise. For examination of the neuro-immune interaction in healthy human volunteers, [^{18}F]FDG PET was applied. Four healthy male volunteers (mean age ± S.D.: 22.3±2.0 years old) were studied with [^{18}F]FDG PET following a transient exercise task to evaluate an acute effect of exercise. Another three healthy volunteers (mean age ± S.D.: 20.0 ± 0.0 years old) were studied with [^{18}F]FDG PET before and after a 4-day-long consecutive exercise task to evaluate a chronic effect of exercise. Linear correlation between NKA and the regional brain metabolism in these volunteers was examined using statistical parametric mapping (SPM) (Fig. 3).

Following acute exercise, positive correlation to NKA was identified in the pre- and post-central gyrus, temporal pole (Brodmann's area: BA38), as well as in the cerebellum. Following chronic exercise, positive correlation to NKA was identified in the extended areas in the frontal cortex (BA6,8,9), temporal pole and inferior frontal gyrus (BA47). These results might suggest the possibility that the neuro-immune interaction is mediated by the temporal pole and inferior frontal gyrus and that the broad areas of the frontal cortex is involved. These results might be in accordance with our hypothesis that NKA changes following exercise is mediated by the cerebral cortex and limbic system (Fig. 3).

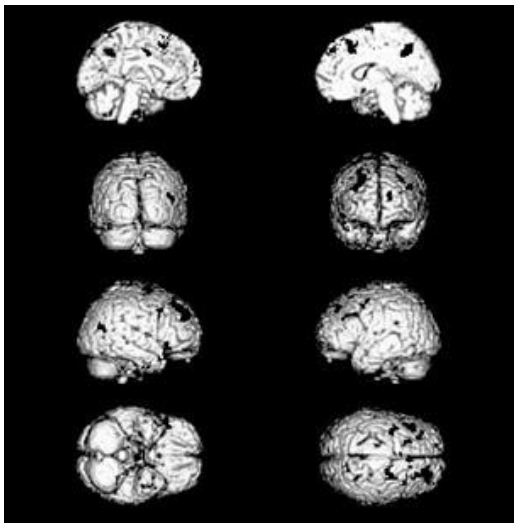

Fig. 3. Brain regions of positive correlation to cellular immunity (Natural killer cell activity) following continuous 4-day-long exercise task in healthy volunteers. The regions include the prefrontal cortex and temporal poles, suggesting the involvement of the brain in exercise-induced immune augmentation. This result was generated using statistical parametric mapping (SPM) software, with height threshold of p = 0.001 and extent threshold of 20 voxel minimum, with no correction for multiple comparisons.

2.3. Functional imaging study on whole-body effects of exercise

In addition, we have attempted visualization of the metabolic alteration at whole body level in healthy male volunteers. Five subjects served as exercise group whose ages ranged from 21 to 23 y (21.8 ± 0.8 years; mean ± S.D.). Another 6 subjects, aged mean 24 ± 5.3 years old were studied as resting control maintaining the same study protocol without exercise. A written fully informed consent was obtained from each subject before the study. This study protocol was approved by the Ethics Committee and Clinical Committee for Radioisotope Studies of Tohoku University School of Medicine. Ergometer bicycle exercise was arranged at 40% and 70% $\dot{V}O_{2max}$ workloads. $\dot{V}O_{2max}$ was measured by intermittent exercise on an ergometer bicycle, and oxygen consumption rate was determined by an automated metabolic unit machine (AE280-S, Minato Co. Ltd. Osaka, Japan). Before the experiment, subjects rested for 20 minutes in a dim lit quiet room. Another teflon catheter was inserted to opposite antecubital veins for [^{18}F]FDG administration. Then, they started ergometer bicycle riding at the speed of 60 revolution/min at both workloads (40% and 70% $\dot{V}O_{2max}$). [^{18}F]FDG was injected through a catheter at 10 min later following exercise task. The radioactivity dose for the exercise group was 38.4 ± 2.2 MBq (mean ± S.D.). After injection, subjects continued to pedal the bicycle for another 30 min, completing a total of 40 min task (Fig. 4).

Subjects lay down in supine position on PET table for measurement. The PET room was kept dimmed and quiet. The scan protocol was as follows: a 3 dimensional (3D) whole-body emission scan (3 min × 9 frames) was performed from knee to the vertex followed by transmission scan (3 min × 9 frames) using a PET scanner (SET2400W, Shimadzu, Kyoto, Japan). The transmission scan (post-injection mode) was performed with a ^{68}Ge/^{68}Ga external rotating line source (370 MBq at purchase). For quantification, both standardized uptake value (SUV) and regional metabolic rate of glucose (rMRglc) were calculated [14].

[^{18}F]FDG uptake was remarkable only in the brain, heart and urinary bladder in the resting subject, while high uptake was visualized in skeletal muscles at exercise state (Fig. 3). Glucose metabolism showed significant increase in the skeletal muscles of the thigh and lumbar/gluteal regions, and was decreased in the brain after exercise task (40% and 70% $\dot{V}O_{2max}$ workloads). A correlation between SUV and rMRGlc was found among organs (i.e., thigh, liver, heart and brain), except in the lumbar/gluteal muscles. A good correlation was observed between SUV and rMRGlc in the brain; however, a non-suggestive correlation was found in the lumbar/gluteal skeletal muscle. The changes in plasma metabolites were as follows: stable plasma glucose concentrations, an increase ($p<0.05$)

plasma lactate concentration at post-exercise condition of 70% VO_{2max} (5.3 ± 2.4 mmol/liter) to compare with pre-exercise condition (0.9 ± 0.2 mmol/liter). The plasma insulin concentration was decreased ($p<0.05$) only at post-exercise workload of 70% VO_{2max} (2.0±0.7 μU/mol) than pre-exercise condition (4.6 ± 1.5 μU/mol). Organ glucose uptake either increased or decreased almost linearly with exercise loads up to moderate workload (70% VO_{2max}). In spite of complexity of energy metabolic controls such as glucose-fatty acid metabolic interaction, aerobic-anaerobic interaction, and involvement of glycogenolysis, exercise-induced organ glucose metabolism were successfully assessed with [^{18}F]FDG PET technique and two analytical approaches. Organ glucose uptake either increased or decreased almost linearly with exercise loads up to moderate workload (70% VO_{2max}), suggesting of homeostatic metabolic control. Semiquantitative method without blood samplings was found useful to estimate [14] (Figs. 4 and 5).

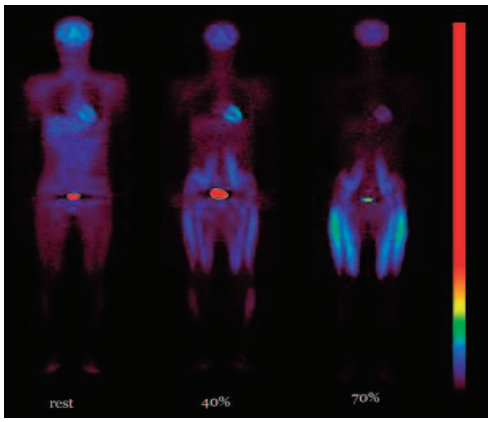

Fig. 4. Whole-body coronal PET images with regions of interest (ROIs) analysis settings. Images are shown at rest, and 40% and 70% VO_{2max} exercise workloads from left to right, respectively. High accumulation of [^{18}F]FDG was observed in the thigh muscles from rest to 40% and 70% VO_{2max} workloads.

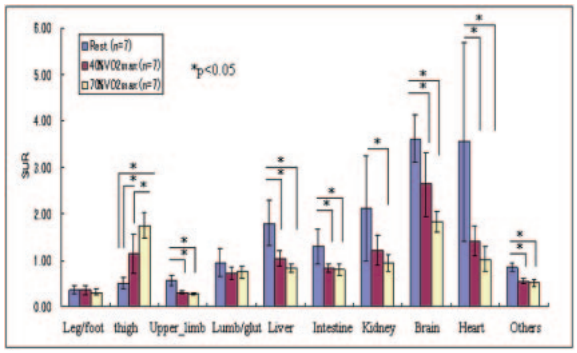

Fig. 5. Diagram demonstrating whole-body averaged regional standardized uptake ratio (SUV) values in the thigh muscles, liver, heart, and brain etc. at rest, 40% and 70% VO_{2max} workloads.

2.4. Neuroimaging for mental care and QOL improvement of patients with sever diseases

Functional neuroimaging techniques such as PET have been used for detecting malignant tumors at early stages and for differentiating malignant from benign tissues. PET has been also used for studies of neurological and psychiatric disorders. Brain PET study in oncology seems to have been exclusively focused on brain tumors thus far. Although there are many reasons that would lead us to believe that a cancer patient's brain is not functionally normal, the use of PET imaging to evaluate the psychological and behavioral aspects of such patients has been rare. Rather, it seems that [^{18}F]FDG brain images of cancer patients have been added to a resting normal control database, based on a belief that a cancer patient's brain without metastasis should be normal. This may be true in terms of a rough anatomical evaluation, but such a belief cannot be firmly held in terms of functional imaging because many cancer patients tend to have psychological abnormality that is sometimes at a subclinical level, as mentioned above. Such a relatively mild abnormality also could be detected by means of functional imaging. Because cancer and cancer treatments have various effects on the central nervous system, the diagnosis of psychiatric symptoms in cancer patients is problematic. Nano-bio-imaging could be used as a supplementary diagnostic tool. Previously, we have proposed the use of [^{18}F]FDG PET in the neuropsychiatric evaluation of cancer patients, and have been performing a series of studies to examine whether the images of a cancer patient's brain are in fact normal or not.

It is now widely accepted that psychological factors are equally important to external factors in disease progression. Some studies have shown that cancer patients with attitudes of "helplessness" and "hopelessness" had a shorter survival period than those with "denial" and "fighting spirit" attitudes. They confirmed the reproducibility of their past study by demonstrating again the negative effect of helplessness, hopelessness and depression. Thus, psychological evaluation and patient care are very important not only for improving quality of life (QOL) but also for prolonging survival. If we suppose a certain psychological disturbance truly exists to the extent of affecting systemic functions of patients, it is also possible for their brain activity to have significant alterations.

In our previous studies, both Japanese and German patients manifested abnormal regional metabolism compared with benign disease patients. Common findings in these studies were hypometabolism in the prefrontal cortex, anterior and posterior cingulate gyri, insular cortex and striatum. These regions were similar to those demonstrated in previous neuroimaging studies of patients with major depression showing commonly repeated findings of hypometabolism in the prefrontal cortex, anterior cingulate gyrus and basal ganglia. Typical findings of hypometabolism are observed in the

anterior cingulate gyrus, prefrontal cortex, lateral prefrontal cortex, basal ganglia as well as in the temporoparietal regions. Thus, considering the similarity to known lesions in major depression, it should be possible to postulate that the regional metabolic reduction in cancer patients starts at very mild stages of depression. Even relatively mild disorders such as adjustment disorder would be accompanied by hypometabolism. The present study also suggests that the depth of depression has good correlation with the regional metabolic reduction in relatively mild stages of depressive disorders, although it seems that such correlation is not evident in major depression.

Thus, our previous findings are summarized as follows:

Point 1) Cancer patients may manifest clearly distinguishable regional metabolic abnormality in the limbic structures and frontal cortex (mainly metabolic decline) measurable by [^{18}F]FDG PET. If cancer patients are included in the so-called normal control database. Also, our results suggest that even in completely healthy volunteers, mild depression and anxiety might affect brain metabolic pattern.

Point 2) This metabolic abnormality seems to be associated with psychological factors such as depression. Chemotherapy and paraneoplastic factors also affect metabolic activity but presumably to a lesser extent based on our preliminary study. Replication with a large number of patients is necessary.

Point 3) Since [^{18}F]FDG PET can detect the effect of relatively mild depression, it might be applicable to the objective evaluation of the psychological aspect of cancer patients as a supplementary evaluation technique. Nowadays, whole-body [^{18}F]FDG PET is a commonly used routine diagnostic procedure. If this technique can be applied to the objective evaluation of QOL in combination with brain PET, we might open a door into a new field of whole-person medicine addressing the physical to spiritual dimensions of a human being.

2.5. Scientific observation of brain functions regarding the efficacy of alternative medicine

As mentioned above, exercise contributes to health promotion and disease prevention. However, in modern society, interest in the therapeutic efficacy of alternative medicine is growing stronger. Not only exercise but also alternative therapy has attracted public attention and, from the viewpoint of evidence-based medicine (EBM, PET can be used for producing supporting data for the efficacy of alternative medicine). PET offers us a means of obtaining physiology data that cannot be obtained by other methods.

We have reported a PET study regarding the effects of aromatherapy. We performed PET examinations using [^{18}F]FDG in 10 women under 2 conditions of “resting” and “aroma patch stimulation”. Monitored autonomic nervous activity showed changes corresponding to the subjects’ autonomic nerve activity of parasympathetic nerve predominance.

3. Nano-bio-imaging study on neurotransmitting functions

3.1. Functional imaging of the histaminergic nervous system

Stress in our daily lives has been associated with the increased rate of various psychiatric disorders such as depression, schizophrenia, and cognitive disorders. To date, we have conducted many studies to elucidate the pathophysiological mechanism of the above-mentioned disorders, putting emphasis on alteration in neural transmission of the histaminergic nervous systems [2]. For this purpose, [^{11}C]doxepin, a tracer of choice for imaging histamine H1 receptors (H1Rs), has been a suitable tool, and increasing evidence has been accumulated regarding the role of histaminergic neuron system in the pathophysiology of these disorders. Histamine H1 receptor binding was measured by using PET and [^{11}C]doxepin, in 10 normal male subjects and 10 patients with schizophrenia [15], major depression [16], as well as in 10 normal female subjects and 12 female patients with anorexia nervosa [17]. In these studies, interestingly, significant reduction in H1 receptor binding was observed in the patients with schizophrenia and major depression while significant increase was observed in the patients with anorexia nervosa (Fig. 6). Further investigation will elucidate the mechanism of these neuropsychiatric disorders in terms of stress responses in the brain histaminergic neuronal system. Thus, PET is very useful for elucidation of pathophysiological mechanism in various disorders [17].

The central histaminergic neuron system modulates a variety of brain functions, especially eating behaviors. We hypothesized that females have higher density of histamine H1 receptor (H1R) in the limbic system than males and that the central H1R is increased in patients with anorexia nervosa (AN). In this study, subjects were 12 female patients with AN, 12 healthy females and 11 healthy males. PET examination was performed with injection of [^{11}C]doxepin. In addition, abnormal eating behaviors, depression, and anxiety of subjects were evaluated with eating attitude test 26 (EAT-26), self-rating depression scales (SDS), and state-trait anxiety inventory (STAI). Binding potential (BP) of [^{11}C]doxepin in females was significantly higher than that in males in the amygdala, hippocampus, medial prefrontal cortex, orbitofrontal cortex, and the temporal cortex. Patients with AN showed significantly higher BP of [^{11}C]doxepin in the amygdala and the lentiform nucleus than control females. In patients with AN, BP of [^{11}C]doxepin in the amygdala and the thalamus significantly negatively correlated with EAT-26 scores. There were significantly negative correlations between BP of [^{11}C]doxepin and SDS scores or STAI scores of

AN in the amygdala, the anterior cingulate cortex, and the orbitofrontal cortex. These findings support the first hypothesis that females have higher density of H1R in the limbic system than males. These results also suggest that patients with AN may have higher expression of H1R in the limbic brain, especially amygdale [17] (Fig. 6).

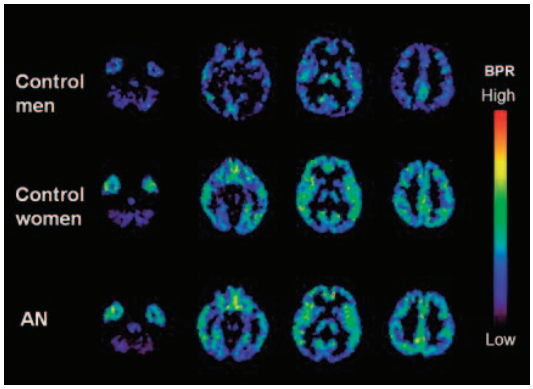

Fig. 6. Brain distribution of [^{11}C]doxepin radioactivity in control subjects and anorexia (AN) patients. Modified from the reference [17].

In addition, PET and [^{11}C]doxepin has been also useful for evaluation of drug-induced side effects and of their mechanism. One of the most frequently used therapeutic drugs for allergies such as seasonal pollinosis (or "hay fever") is a H1R antagonist (antihistamine). There are many available antihistamines but some of them have sedative side effects. Therefore, it is important to develop an objective and reliable method for measuring the strength of such sedative side effects [2]. To date, we have studied the mechanism of functional suppression in signal transmission through the H1Rs in the brain. Usually, antihistamines are used to suppress the actions of mast cells in the peripheral blood and to control allergic reactions. However, some of these drugs may enter the brain and suppress the signal transmission of intra-cerebral H1Rs. As a result, it becomes difficult to maintain arousal (sedative effects), and sometimes these drugs might cause us to make mistakes during work or while driving, resulting in decreased work efficiency or traffic accidents. Considering such a background, objective measurement of the sedative effects of these drugs becomes very important.

We have succeeded in quantifying the strength of the sedative effects of antihistamines in terms of H1R occupancy (rate) in the brain using PET, and we have measured this clinically. Previously, investigators have performed macroscopic behavioral techniques such as measurement of psychomotor performance including psychomotor speed and accuracy as well as the measurement of subjective sleepiness using many volunteer subjects to evaluate drug sedative effects. Recently, we have conducted a clinical test to evaluate the sedative profiles of bepotastine besilate, a new antihistamine developed in Japan [18]. The basic pharmaceutical classification of this antihistamine has been regarded as "a mildly-sedative antihistamine". We succeeded in obtaining supporting data regarding this classification using PET [18] (Fig. 7).

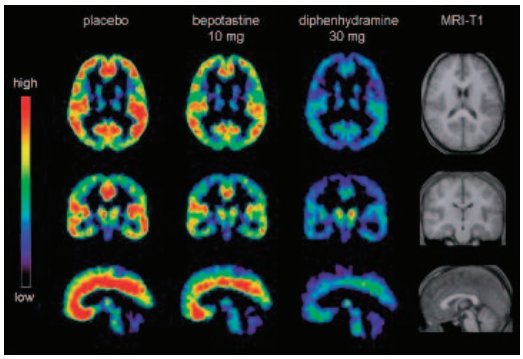

Fig. 7. Binding potential ratio (BPR) images of [^{11}C]doxepin in the human brain. BPR of [^{11}C]doxepin was measured in healthy subjects using PET after oral administrations of placebo (left), bepotastine (10 mg) or diphenhydramine (30 mg) for each treatment condition were compared. Modified from the reference [8] by courtesy of Blackwell Publishing Company.

From the viewpoint of cognitive neuroscience, the category of cognitive function (psychomotor performance) measured in antihistamine studies is mainly "vigilance and attention". The sedative side effects of antihistamines have been recognized to be potentially dangerous in our daily tasks such as car driving, but the mechanism underlying these effects has not yet been elucidated well. We, therefore, attempted to elucidate the brain mechanism of impaired performance by using car-driving simulator and [^{15}O]H_2O PET [19]. We examined regional cerebral blood flow (rCBF) responses during a simulated car-driving task following oral administration of d-chlorpheniramine using [^{15}O]H_2O PET. Results of performance evaluation revealed that a part of driving performance (lane deviations) significantly increased in the d-chlorpheniramine condition compared with the placebo condition while Subjective sleepiness was not significantly different between the two drug conditions. In addition, brain imaging analysis suggested that d-chlorpheniramine tended to suppress the regional brain activities associated with visuo-spatial cognition and visuo-motor coordination [19] (Fig. 8).

Thus, nuclear medicine technique is very useful for objective evaluation of intensities and mechanism of brain effects of various drugs. These data can be used for development of new drugs with reduced side effects.

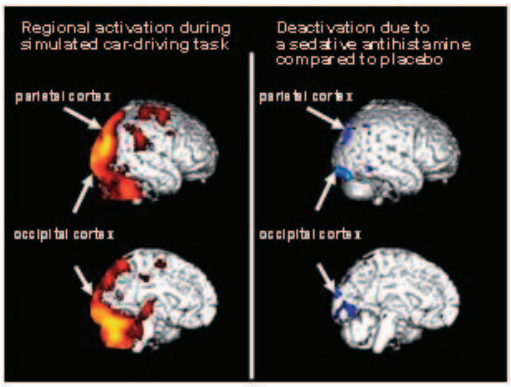

Fig. 8. Results of PET analysis demonstrating regional activation during active driving compared to the resting (yellow & red), and regions with diminished activation under the influence of sedative antihistamine (blue). Modified from the ref. [19] by the courtesy of John Wiley & Sons, Ltd.

3.2. A Study on image analysis and early diagnosis of dementia including Alzheimer's disease

Dementia study has become a very important in our modern society. As for PET imaging of Alzheimer's disease (AD), it has been widely recognized that decreases in regional brain metabolism in the posterior cingulate gyrus and temporal-parietal corticies are typical patterns of Alzheimer's disease, which can be visualized with [^{18}F]FDG. In such demented brains, many nerve cells are already destroyed and the cell population in the brain is decreased, resulting in considerably decreased nervous activity. It has been thought that the neuronal damage is associated with accumulation of β-amyloid protein in the brain, which may precede massive neuronal death. Then, an early diagnosis of AD can be realized if we use a proper tracer specifically binding to β-amyloid proteins in the brain of AD patients. This technique has been called "amyloid imaging". At Tokoku University, we performed the first clinical examination of Alzheimer's disease employing a new tracer, [^{11}C]BF-227, which can be used for direct imaging of amyloid deposits in the brain. Currently, we are establishing a suitable pharmacokinetic model for quantification of β-amyloid deposits in the brain of Alzheimer's disease patients observed by PET and [^{11}C]BF-227 (Fig. 9) (a collaborative study with Professor Y. Kudo of the Tohoku University Innovation of New Biomedical Engineering Center, Professor K. Yanai of the Department of Pharmacology, Graduate School of Medicine, and Professor H. Arai of the Departments of Gerontology and Geriatrics and Alternative and Complementary Medicine, Graduate School of Medicine). The Division of Cyclotron Nuclear Medicine is in charge of the analysis and construction of a useful model in studies of [^{11}C]BF-227 data as well as enforcement of further clinical studies in all laboratories [20] (Fig. 9).

We succeeded in visualizing the clear contrast between "healthy elderly volunteers" and " patients with Alzheimer's disease" using a cerebellar ratio of standardized uptake value (SUV), a simplified semi-quantitative index for clinical diagnosis [20]. We can also collect numerical clinical data (arterial blood samples). Linearization of the data, such as with the use of the Logan method (Fig. 9c), was applied, and it was confirmed that adequate values could be obtained. We can also use the data of time-activity curve in plasma (pTAC) and time-activity curve in tissue (tTAC) obtained through sequential blood sampling and dynamic PET scanning.

Furthermore, at the Cyclotron and Radioisotope Center and in collaboration with Professor K. Yanai, we are using another novel tracer, [^{11}C]donepezil, which is a radio-labeled donepezil, to evaluate neural transmission in an acetylcholine nervous system. In Alzheimer's disease, it is well known that signal transduction in an acetylcholine nervous system is disordered. As a result of our recent study, it was confirmed in the brain of an Alzheimer's disease patient that the number of binding sites of donepezil decreased markedly [21]. We plan to observe whether we can use this [^{11}C]donepezil as a marker for evaluating therapeutic efficacy of treatment using acetylcholine esterase inhibitors of Alzheimer's disease in the future. In addition, we are in charge of the construction of a calculation model regarding this tracer, and analysis is now under way.

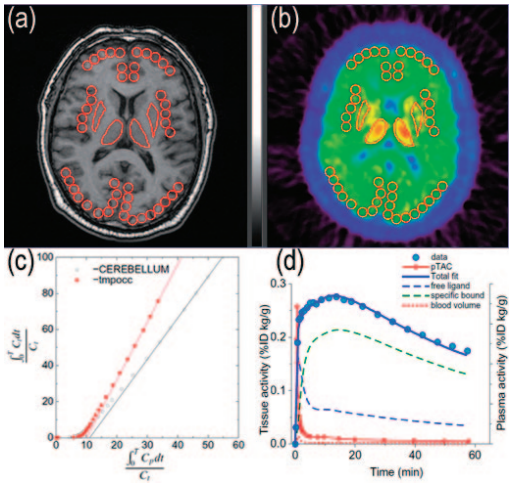

Fig. 9. Examples of pharmacokinetic analysis showing basic procedures of quantification. Regions of interest (ROIs) defined for various brain structures in co-registered MR (a) and PET (b) images. Example of quantification using graphical analysis (c). Time activity curves in plasma and brain tissue for compartmental model analysis (d).

4. Research and Development of Software and Hardware for Cancer Detection

4.1. Development of a new positron emission system (positron emission mammography: PEM) for exclusive use in high resolution diagnosis of breast cancer

In recent years, malignant tumors (cancer) have become the leading cause of death in Japan. Especially, breast cancer is one of the leading causes of death among Japanese women. This type of cancer can be treated relatively easily when it is detected in early stages. The highest incidence of breast cancer is observed among those in their thirties and forties. The high incidence among these age groups might considerably affect the performance and achievement of our society because women in these generations usually play important roles in their families and working places. Therefore the research and development for early detection of breast cancer has been very important for the society.

Recently, imaging techniques such as X-ray mammography and/or ultrasonic echography have been shown to be useful for the detection of breast cancer. However, these modalities provide only morphological information. On the other hand, younger women or women without a history of breastfeeding tend to develop mammary gland tumor. In some cases of X-ray mammography, a developing mammary gland produces shadows on mammography images, making detection of breast cancer difficult.

PET with [^{18}F]FDG has been used for whole-body diagnosis of various cancer, which can provide functional information on cancerous tissues including the degree of malignancy, as reflected by the level of accumulation of [^{18}F]FDG tracer. However, various problems still remain such as limited spatial resolution and motion artifacts due to respiration movement, as well as high costs for device installment and maintenance. In measuring events of the coincidences of positrons, a larger distance between detectors, as seen with PET scanners, for whole-body imaging limits spatial resolution. In order to compensate these disadvantages, we have started development of a positron emission mammography (PEM) scanner dedicated for the local diagnosis of breast cancer.

The basic concept of PEM development consists of the following factors: compact radiation detectors, high-speed electronic circuit, and lower cost of equipment. Finally, the PEM scanner is expected to achieve a high spatial resolution of about 1 mm, which is a quarter of the resolution of conventional PET scanners. Regarding the radiological dose to be needed for examination would be low as much as 20 MBq, approximately one tenth of the dose of conventional PET scanners. Cost performance is also an important issue, and PEM can be developed with the costs of one fifth of the conventional PET scanner.

In our new scanner, a new crystal of praseodymium doped “lutetium aluminum garnet” (LuAG) is used as novel scintillators. This new scintillator has some advantages such as a short fluorescence lifetime, a low manufacturing cost and a high light output. The image mapping system is used for diagnosis of the crystal. The system consists of a personal computer, the core with amplifiers, analog-to-digital converters and the dark box for the scintillation measurement (Figs. 10 and 11). The system is set up in a personal computer rack. A flat panel photomultiplier tube (PMT), H8500, made by Hamamatsu photonics, is used for the scintillation mapping. The mapping method is based on the center of gravity calculation. The system has the gain correction function for the variety of the multi anode outputs from a flat panel PMT. The system can measure the energy spectrum of the scintillation.

Presently, we are still in the process of developing a PEM scanner, and we will also fabricate a prototype PEM scanner for commercial use. This scanner will be used for detecting smaller tumors, and it will be cheaper and smaller than the conventional PET scanner. We are aiming for a spatial resolution of <1-2 mm, and the price will be 20% lower than that of the conventional PET scanner.

The PEM simulator is set up in a personal computer rack and a worktable and used for the diagnosis of the crystals and the PMTs. Last year, we developed several test devices and a PEM simulator. One of these devices is a block detector device. This device is constructed from a flat-panel photomultiplier tube (FP-PMT), an inorganic scintillator crystal array, an aluminum case and a signal processing circuit. The FP-PMT is a multianode PMT with 16 x 16 anodes and has positional sensitivity. The PEM simulator is a testing and an evaluating system of the PEM scanner. The simulator has four block detector devices, high voltage supply units for PMTs and signal processing units. The latter consists of an analog amplifier, an analog to digital converter, Field Programmable Gate Array (FPGA) devices and communication interface for PC (Fig. 11). Currently, evaluation of a prototype scanner is undergoing using various phantoms (Fig. 12).

In summary, this project is now in progress steadily for developing the prototype PEM scanner. Because the PEM simulator was developed with other scintillator, so we are trying to optimize the performance after being equipped with the new LuAG scintillator. And we will assemble and test larger size LuAG scintillator crystal block arrays. If there are no gantry bed then we can not use for the clinical. And we will develop additional software for an image reconstruction, a gantry control, a registration of patients and so on. Finally when this project is finished, we hope that a new possibility will be opened to the breast cancer diagnosis (in collaboration with Prof. Masatoshi Itoh [a specially approved visiting professor of Tohoku University, CYRIC], Professor Mamoru Baba [a professor in emeritus] and Assoc. Prof. Yoshikawa Akira at Division of Physical Process Design, Institute of Multidisciplinary Research for Advanced Materials, Tohoku University).

Fig. 10. Left is the PEM simulator. The PEM simulator is a subset of the PEM scanner and used for the diagnosis of the crystals and the PMTs. Right is a close up of the detector units.

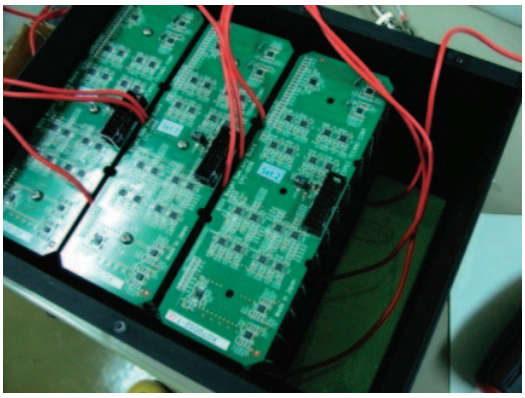

Fig. 11. Opened cover of the PEM detector unit shows an aluminum case, an inorganic scintillator crystal array and electronic circuits for FP-PMTs.

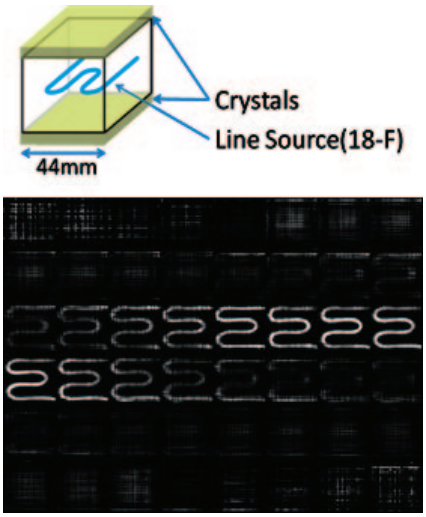

Fig. 12. Geometry of the measurement of a positron line source (TOP) and the measured images of this line source (BOTTOM).

4.2. Development of a whole-body automated diagnostic system

In recent years, malignant tumors (cancer) have become the leading cause of death in Japan. Against this background, the Japanese government has introduced a new law to promote the improvement in the quality of cancer diagnosis and treatment since 2007. In general, cancer examination using PET with [^{18}F]FDG has been conducted as a whole-body scan. Since the number of patients is increasing, the burden on radiologists might be also increasing. If we can develop an automated diagnostic program by simulating the cognitive tasks performed in the brain of radiologists, we may be able to use the outputs from the diagnostic system as supplementary information for the diagnosis. We are currently examining the possibility of making such a system (in collaboration with Prof. Keizo ISHI, Department of Quantum Science and Energy Engineering, Graduate School of Engineering, Tohoku University).

4.3. Study of image information engineering

Development of a novel image processing software is also important, such as an efficient software for co-registration of MR and PET images and CT and PET images. We have been also working in this field of information science. We have succeeded in algorithmic development to accelerate calculation speed to about 10 times while maintaining the precision of image processing. It is expected that the application of this method can shorten working hours for image processing. In addition, we are also conducting a study regarding image reconstruction and scatter correction.

5. Summary

The potential for further application of nano-bio-imaging technology using tiny amount of radiopharmaceuticals is clear. We should also look to develop nano-bio-imaging by continuing collaboration across the various fields of medical science, pharmaceutical science, engineering, information engineering, etc.

Tokoku University has started "a molecule imaging education course" for master and doctoral students at the Graduate Schools of Tohoku University, under the auspices of the "Section for molecule imaging research and education", a part of the whole-school organization established in 2006 (in collaboration with the National Institute of Radiological Science, Molecule Imaging Research Center). In this research and education program, the Cyclotron and Radioisotope Center provides education and training in molecule imaging. Here we can promote the training of specialist personnel while performing maintenance of the various equipment needed to further promote active research. Nano-bio-imaging is essentially an interdisciplinary

field and its development requires dynamic interaction between young researchers. There is considerable potential for nano-bio-imaging techniques using minimal-dose radiopharmaceuticals and the interaction and collaboration of young researchers having a wide variety of academic backgrounds will help to achieve this. The author hopes that the present global COE program can offer an ideal environment for such fruitful interactions.

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