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The usefulness of radiotracers to make the body biochemically transparent

Review Article

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Summary. Radioactive isotopes are uniquely applicable to observe reactions or circuits of reactions at the molecular level without disturbing the system being studied. The advent of molecular imaging modalities, particularly positron emission tomography (PET), is a major breakthrough for the visualisation and quantitative assessment of cellular and molecular processes occurring in living tissues. The recent development of animal PET scanners that offers 2-mm resolution and is tailored to laboratory rodent models, has made a further great impact on *in vivo* biochemistry. With these live-imaging modalities at hand, radiotracer-based technologies allow to look directly at biochemical distribution and interaction processes. Tremendous progress made in radiotracer chemistry, primarily in carbon-11 and fluorine-18 radiochemistry, and in the design of imaging devices strengthens the usefulness of radiotracers in nuclear medicine and drug research and development and opens exciting opportunities for new applications, e.g., in food science.

Keywords: Radiotracer – Positron emission tomography – Molecular imaging

Introduction

Stable and radioactive isotopes are uniquely applicable to observe reactions or circuits of reactions at the molecular level without disturbing the system being studied. In chemistry and biochemistry the ideal tracer has the same chemical properties as the molecule it replaces and undergoes the same reactions but can at all times be detectible and quantitatively assessed. George de Hevesy was the first investigator to use an isotope in metabolic studies; he explored lead transport in the bean plant with a natural radioisotope of lead (de Hevesy, 1940). By measuring the radiolead accumulated in various portions of the plant he was able to quantify the uptake of lead in them. These early studies were limited to the few available naturally radioactive isotopes. For his concept of radioactive indicators, George de Hevesy received the Nobel Prize in 1943 (Stoecklin et al., 1995). Through a series of brilliant experiments with stable isotopes by Rudolf Schoenheimer and David Rittenberg in the 1930s the foundations to the understanding of many basic cellular processes were laid (Schoenheimer and Rittenberg, 1935, 1938). Schoenheimer and colleagues used deuterium to replace some of the hydrogen atoms in molecules of fat, which he fed to laboratory animals. On analysing the body fat of rats four days later, they found that there was a constant changeover in the body between stored fat and fat that was used. The investigation of the metabolism of fatty acids and sterols is facilitated by the great stability of the carbon-bound hydrogen, which has been shown does not exchange with the hydrogen of water. Furthermore, Schoenheimer and colleagues used the isotope nitrogen-15 to label amino acids and again found that component molecules of the body are continually being broken down and built up (Schoenheimer, 1942). So, tracer studies in the 1930s and 1940s showed that cells are in a dynamic metabolic state, that compounds necessary for growth and stability are constantly 'turning over'. Today, these basic rules of biochemistry are a given concept that is obvious to us all. Radioactive isotopes are more easily detected than non-radioactive ones, such as deuterium or nitrogen-15; therefore, when the radioactive isotopes of various atoms commonly occurring in organic molecules became widely available the radiotracer techniques developed rapidly. Particle accelerators and in particular cyclotrons as well as reactors were used to

produce a large variety of artificial radioisotopes. Whereas radioisotopes made in a reactor are usually neutron rich, cyclotron-produced radioisotopes are mainly neutron deficient. The classification into these two groups of radioisotopes is important because of the different nuclear-physical properties of the two groups of radioisotopes and the different applications they are predestined for. The cyclotron is required for the production of radioisotopes such as carbon-11 which is used for positron emission tomography (PET), and a cyclotron forms thus an integral part of a PET centre. Carbon-11 has a 20.4 min half-life and decays by positron emission. The short half-life and body-penetrating radiation resulting from positron decay provides the potential to image biochemical reactions in the living body with a low radiation dose.

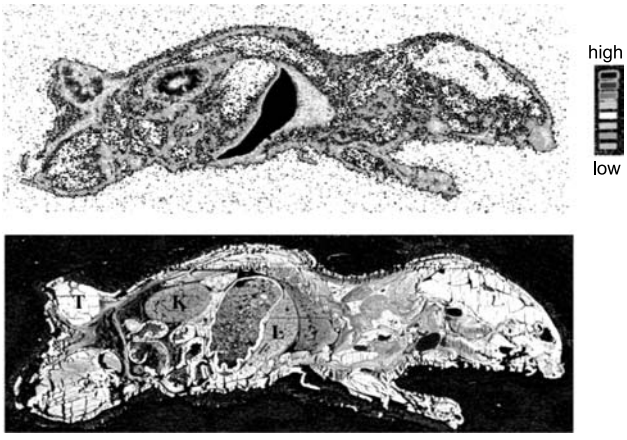

Fig. 1. Whole-body autoradiography in a tumour-bearing rat after administration of a fluorine-18 labelled neurotensin derivative (top), demonstrating uptake in the tumour (*T*) and the excretory organs, i.e., kidney (*K*) and liver (*L*), compared to a photographic image of the slice (bottom)

Imaging modes

Molecular imaging aims at visualizing cellular and molecular processes occurring in living tissues (Weissleder and Mahmood, 2001; Herschman, 2003). Imaging is becoming a fundamental technology of integrative biology. Many live-imaging modalities have been developed, such as computed tomography (CT), magnetic resonance imaging (MRI), optical imaging (fluorescence, bioluminescence), single-photon emission computed tomography (SPECT), and PET. Some of them depict primarily anatomical or morphological information. Radiotracer-based technologies allow insights directly at biochemical distribution and interaction processes. A most useful research tool to complement *in vivo* imaging techniques is autoradiography (Bergstrom et al., 2003). Today, the recording of radioactivity is typically made with imaging plates. *Ex vivo* autoradiography of selected organs or whole animals gives images of a radiotracer's distribution with high resolution, however, only for one time point after administration of the radiotracer. Autoradiography performed as an *in vitro* or *ex vivo* binding technique is widely used to assess binding of biomolecules and drugs to receptors or transport molecules (Fig. 1). *In vivo*, radiotracers can be imaged with specifically sensitive scanners or cameras in various configurations. In the mid 1950s, Ter-Pogossian promoted the idea that in spite of their short half-lives the positron-emitting radionuclides, such as carbon-11, offered an attractive method for the regional study of metabolism due to their commonality (Ter-Pogossian, 1966). Since the early 1970s, PET has been used as a research tool, and in more recent years has shown increasing potential for its application to clinical medicine. PET is unique because it produces images of the body's biochemistry. It makes the body biochemically transparent and, particularly, allows to look at the protein level, where transporters, receptors, and enzymes act (Phelps, 2000). The recent development of animal PET scanners that offers 2-mm resolution and is tailored to small laboratory animal rodent models has made a great impact on *in vivo* biochemistry (Chatziioannou, 2002; Lewis, 2002).

PET in nuclear medicine

Nuclear medicine is, by definition, the use of radionuclides to diagnose or treat disease. Choice radionuclides are used to prepare radioactive drugs (radiopharmaceuticals) that are administered to the patients. With the advent of PET it became possible to quantitatively measure regional activities of a positron emitter labelled molecule with high sensitivity. This extended nuclear medicine to *in vivo* biochemistry. Inherent in the application of radiotracers is the concept of molecular targeting (Britz-Cunningham, 2003). Although PET cannot directly analyze biomolecules, it uses radiotracers to

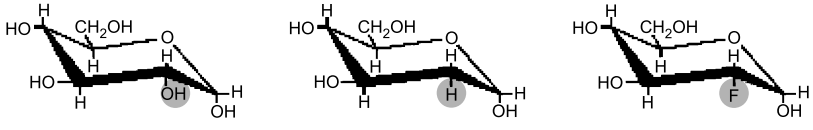

Fig. 2. Structure of D-glucose, 2-deoxy-D-glucose and 2-fluoro-2-deoxy-D-glucose (FDG)

characterize key steps of diagnostic relevance in a biological process. The approach is best exemplified by radiofluorinated deoxyglucose, 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F] FDG). [^{18}F] FDG has become the most commonly used molecular imaging probe for PET studies. It is a substrate of glucose transport into cells and subsequent hexokinase-mediated phosphorylation, resulting in metabolic trapping of the radiotracer in cells with enhanced glucose metabolism (Fig. 2). Many contributions confirmed the diagnostic value of PET with [^{18}F] FDG in various neoplasms, such as lung, breast, oesophageal, pancreatic and colorectal cancer, malignant melanoma, and Hodgkin's and non-Hodgkin's lymphoma. In addition, PET with [^{18}F] FDG is increasingly applied for the diagnosis of neurodegenerative disorders, such as Alzheimer's disease (Fig. 3). Furthermore, abnormalities in brain neurotransmitters are associated with many neurological and psychiatric disorders. PET enables the direct measurement of components of the neurotransmitter systems in the living brain. PET radiotracers can be used to determine the various neurochemical components, such as the neurotransmitters and precursors, receptors and transporters. Receptor imaging agents, i.e. 6-[^{18}F]fluoro-L-3,4-dihydroxyphenylalanine ([^{18}F] FDOPA), are a good example to mention that labelled biomolecules have to meet a number of criteria to be useful for PET studies (Barrio et al., 1990). An ideal labelled receptor antagonist or agonist should reach the target organ and bind there only to the receptor of interest. On its way to the target, the intravenously injected tracer should bind as little as possible to plasma proteins and blood cells. Furthermore, metabolism should as slow as possible. The binding affinity to the receptor and the kinetics should be appropriate to achieve sufficient target/non-target ratios and to allow estimation of binding data and receptor densities (Fig. 4). PET using appropriate labelled amino acids and derivatives is an established *in vivo* means of measuring amino acid metabolism in tissues such as the brain and tumours. The significance of these tracers is their application to measuring amino acid transport and/or protein synthesis rates in tissues. The examples given above are meant to illustrate the important role PET is playing in the medical specialties of oncology (Jerusalem et al., 2003), neurology (Jacobs et al., 2003), psychiatry (Parsey and Mann, 2003) and cardiology (Schwaiger and Bengel, 2002). In Table 1, several biochemical processes addressed by PET in oncology are listed. Molecular imaging with radiotracers also gives reliable indications as to treatment response and will thus play an important role in patient management.

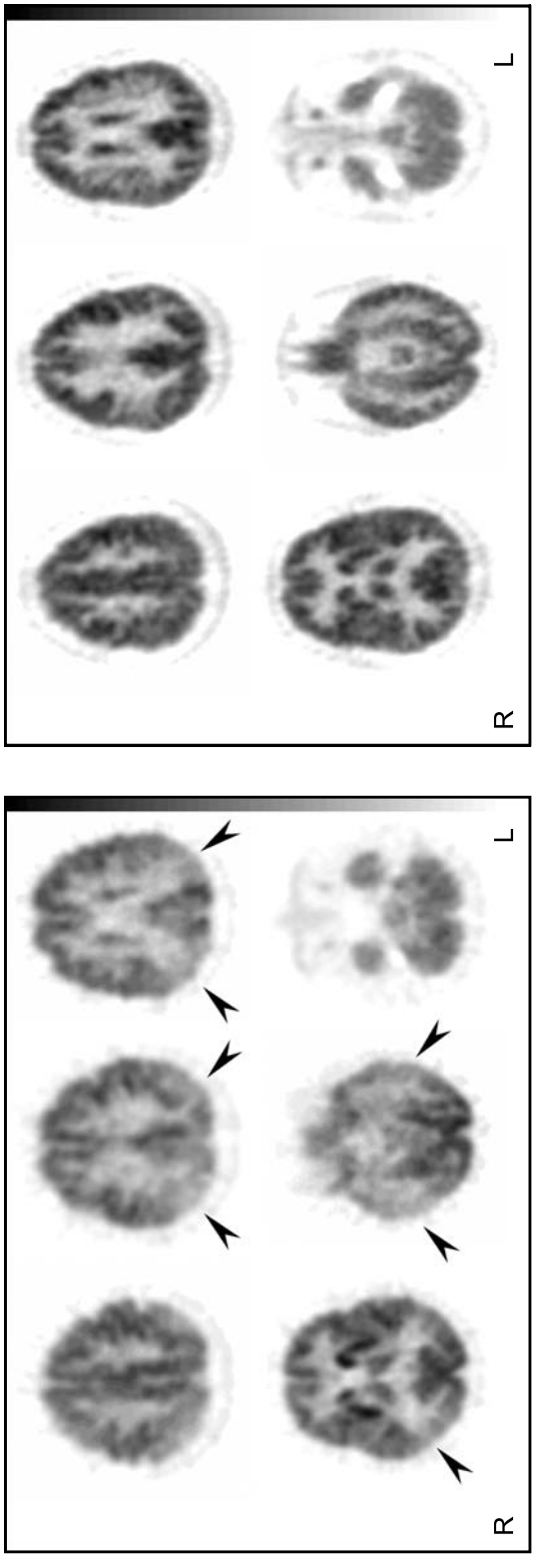

Fig.3. PET images of human brain in a patient with Alzheimer's disease (left) and normal control (right) following intravenous injection of 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F] FDG). Functional lesions are indicated by arrows

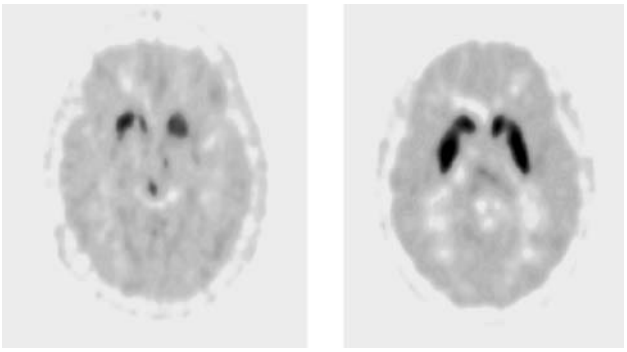

Fig. 4. PET evaluation of dopamine synthesis in human brain in a patient with Parkinson's disease (left) and normal control (right) following intravenous injection of 6-[^{18}F]fluoro-L-3,4-dihydroxyphenylalanine ([^{18}F] FDOPA)

Table 1. Several biochemical processes addressed by PET in oncology

Glycolysis
Active transport
Cell proliferation
Multidrug resistance
Hypoxia
Apoptosis
Angiogenesis
Binding to tumor-associated antigenes and receptors
Other metabolic processes, e.g., accumulation of [^{11}C]acetate in prostate cancer
Gene therapy monitoring

PET in drug research

The development of a new drug is expensive and time-consuming. Recently, there has been tremendous interest in applying PET technology to drug research and development (Maclean et al., 2003; Volkow et al., 1999). Significant improvements in the imaging technology now permit a wide range of PET studies in mice and rats, using compact, relatively low-cost, dedicated small-animal PET scanners. Particularly the availability of positron-emitting isotopes of carbon and fluorine, namely carbon-11 and fluorine-18, allows the preparation of labelled drugs with chemical composition close or identical to the actual drug substance. The radiolabelled drug substance, where carbon-11 or fluorine-18 replaces the equivalent atom in the ''cold'' compound, is used to investigate *in vivo* biodistribution and other pharmacokinetic parameters of the specific compound. PET is sensitive enough to detect trace amounts of the labelled compound and can easily provide information about what happens to a drug over time (its pharmacokinetics). The direct measurement of drug pharmacokinetics depends on the ability to incorporate the short-lived positron emitters appropriately into drug molecules. PET also can measure drug effects on regional biochemical function and can successfully address pharmacodynamic issues. Predominantly, the effect of drugs on glucose metabolism using FDG has been studied. In this case, a radioactive biomarker represents the PET probe instead of the labelled equivalent of the drug under investigation. The usefulness of receptor occupancy measurements with PET using carbon-11 and fluorine-18 radioligands for the development of drugs that target receptors is generally acknowledged. PET is also ideally suited for studies of addiction (Lindsey et al., 2003; Volkow et al., 2003). So, in short, PET is increasingly applied in different areas and at different stages of drug development.

PET radiotracers

Since radiotracers are essential and decisive to address a given biochemical question with PET, the access to radiotracers needs special consideration. Their synthesis starts with the production of the positron emitters, in particular the short-lived ''organic'' isotopes carbon-11 and fluorine-18, in a cyclotron. Because of the particularities, such as very low amount and concentration (carrier-free radionuclides), high starting radioactivity, penetrating gamma radiation, and working against time (20.4 and 109.8 min half lives), a new technology of fast and usually automated chemistry was developed in order to chemically integrate the radionuclide into the biomolecule to be labelled. The position of the label must be considered in relation to the metabolic pathway of the compound. In the preparation of PET radiotracers for human application the final product has to be sterile, pyrogen-free and suitable for intravenous injection. The pharmaceutical requirements on Good Manufacturing Practice (GMP) have to be met. All the factors described above make the development of a new radiotracer challenging and time-consuming. Because of the great capability and potential of PET, this effort is worthwhile.

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