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Metabolite analysis in positron emission tomography studies: examples from food sciences

Review Article

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Summary. Substances of various chemical structures can be labelled with appropriate positron emitting isotopes and applied as tracer compounds in PET examinations. Using dynamic data acquisition protocols, time-activity curves of radioactivity uptake in organs can be derived and the measurements of tissue tracer concentrations can be translated into quantitative values of tissue function. However, analysis of metabolites of these tracers regarding their nature and distribution in the living organism is an essential need for the quantitative analysis of PET measurements. In addition, metabolite analysis contributes to the interpretation of the images obtained as well as to the identification of pathological changes in metabolic pathways. This paper reports on representative examples of radiolabelled compounds which might be of importance in food science (e.g., amino acids, polyphenols, and model compounds for advanced glycation end products (AGEs)). Typical procedures of analysis (radio-HPLC, radio-TLC) including pre-analytical sample preparation are described. Specific challenges of the method, e.g., trace amounts of radiolabelled compounds and the influence of the often very short half-lives of positron-emitting nuclides used are highlighted. Representative results of analyses of plasma, urine, and tissue samples are presented and discussed in terms of the metabolic fate of the tracers.

Keywords: Positron emission tomography – Metabolite analysis – Tracer – Amino acids – Advanced glycation endproducts – Polyphenols

Introduction

Positron emission tomography (PET) is currently the most useful imaging technique for non-invasive measurement of drug pharmacokinetics regionally in a variety of tissues. Over the past decade, PET measurements have provided many critical insights about the tissue distribution of several classes of drugs; neuroleptics, antimicrobials, antineoplastics, etc. PET measurements can be performed after any route of drug administration, intravenous, inhalation or oral, however, intravenously administered drugs have been the most extensively evaluated. In food sciences, particularly, studies of orally administered drugs are clearly of great interest; however, formulation issues have precluded widespread applications in these areas. PET involves the acquisition of physiologic images which represent the distribution of radioactivity related to positron emitting nuclides in the volume of investigation. PET imaging is unique in that it shows the chemical functioning of organs and tissues, while other imaging techniques, such as X-ray computed tomography (CT) or magnetic resonance imaging (MRI), majorily show structure. PET combines the important characteristics of: (a) absolute quantitation, (b) short half-lives of PET radionuclides permit repetitive, longitudinal studies before and after interventions, (c) resolutions of <2.5 mm can be achieved with dedicated small animal PET cameras in almost any tissue of the body, (d) procedures developed in animal studies are readily applied to human studies and most importantly, (e) almost any drug can be radiolabelled with carbon-11, nitrogen-13 or fluorine-18 and thus can serve as a PET tracer (Phelps, 2000; Herschman, 2003). Currently, PET is the only quantitative and noninvasive technique for making tissue pharmacokinetic measurements non-invasively in humans.

Using appropriate radiotracers it is possible to study such different objects as physiological processes and pathological changes in the human body, pharmacokinetics of newly developed drugs or modes of action of

food ingredients by means of PET. In each case, however, one has to keep in mind for the interpretation of the images that the measured signals alone do not allow any conclusion about the chemical compounds that stand behind the radioactivity. In the living organism PET tracer substances, as any other xenobiotics or drugs, may undergo biotransformation reactions including oxidation, hydrolysis or decarboxylation as well as several conjugation processes (Williams, 1959; Silverman, 1995; Testa, 1995, Löffler and Petrides, 1998; Fischman et al., 2002; Hammond et al., 2003). As a result, a great number of chemically different species may be formed whereas the amount of the parent compound decreases. The measured signal in a PET scan therefore reflects the total of all the compounds formed, provided that they still bear the radioactive label. Metabolic transformations of the tracer compounds may be of interest for PET examinations in different concerns. First, since it seems clear that metabolic products of a tracer may show substantially different distribution patterns in the body compared to the parent compound, knowledge about these conversions is essential for the elementary understanding of the radioactivity distribution throughout the body. As an example, the metabolism of 2-fluoro-2-deoxyglucose (FDG) beyond FDG-6-phosphate has been examined in different organs of rats *ex vivo*. Significant inter-tissue differences in the extent of the further metabolism have been shown and a tissue-dependent relative accumulation of each metabolite reflecting the metabolic and regulatory characteristics of each organ has been demonstrated (Southworth et al., 2003). The knowledge about biochemical nature and occurrence of the diverse metabolic products can therefore lead to deeper insights into normal physiological as well as pathological processes by means of PET. Metabolic abnormities might be recognized by a differing distribution pattern and hints for the reasons might be derived. Furthermore, one has to consider any metabolic changes of the tracer in blood and tissues, whenever physiological parameters, such as metabolic rate or receptor density should be derived. For this purpose, mathematical models have to be developed which describe the relationships between the measurable data and the physiological parameters affecting tracer uptake and conversion. These models require profound information on the underlying processes of biochemistry and distribution of the tracer, which can hardly be obtained without examination of its metabolisation (Melega et al., 1991; Carson, 2003). Additionally, tracer metabolism markedly affects the arterial input function used for a quantitative analysis of the images (Ishiwata et al., 1998; Oikonen, 2004). In this context, metabolite analysis in plasma is an essential prerequisite to determine the exact arterial input function for quantitative PET measurement. In order to clear up the metabolic changes of a substance of interest, be it just to understand the general ways of distribution and excretion or, more sophisticated, to derive any kind of biochemical parameters, one has to establish appropriate experimental models, that would allow the simulation of the typical reactions *in vitro* (e.g., in cell cultures) or *in vivo* (in laboratory animals). Using this experimental approach it is possible to obtain information about processes inside organs or tissues that can obviously not be evaluated in man. However, one has to consider carefully inter-species differences when drawing conclusions from model investigations to human studies. Depending on the study design different steps of metabolic analysis might be necessary. A relatively simple task is only to determine the amount of the unchanged portion of a parent compound in plasma, which is needed to obtain the corrected arterial input function for the quantitative analysis of PET images. In this case the analytical procedure is focused on one chemical species in a comparative simple environment that has to be separated from the rest of labelled compounds. A further challenge for the separation as well as for the preanalytical procedures is the development of methods, which allow the identification of unknown metabolites not only in plasma but also in different organs or tissues. However, very short half-lives of PET nuclides have to be considered for all analyses.

Methods applied for metabolite analysis in PET measurements

In comparison to non-radioactive methods of drug metabolite analysis there is one point in PET investigations that represents some kind of simplification for the analytical procedures: Only those compounds are detected in a PET scan that bear the radioactive label. Therefore, only the labelled portion of biochemical species is of any analytical interest and can advantageously be detected by radioactivity detectors of different type. In general, the analytical methods applied are the same as in classical drug development and analysis. These are mainly chromatographic methods among which in recent years HPLC might be the most widely applied. HPLC with radioactivity detection, also referred to as ''radio-HPLC'', allows high-speed, high-precision analyses. On-line radioactivity registration is usually achieved by flow detectors. The equipment with special PET flow cells in connection with appropriate detector geometry allows detection limits

ranging from 0.3 to 1 Bq/μL (Maziere et al., 1993; Takei et al., 2001). Several techniques of separation, as normal phase, reversed phase, ion-exchange, ion-pair or size exclusion, are applicable depending on the chemical structures of interest. The major drawbacks of HPLC are the relatively high costs for hardware and consumables as well as the limited sample-throughput which is of importance for large series of samples in connection with short-lived isotopes. In addition, careful sample pre-treatment is necessary to prevent failure of analyses, e.g., clogging of the columns, by the sample matrix.

TLC techniques, in comparison, also require pre-treatment procedures although they are usually more robust against minor impurities in the matrix. Moreover, they allow simultaneous processing of large numbers of samples. Radioactivity registration is accomplished either by direct measurement using a highly-sensitive TLC scanner or by indirect detection via radiosensitive imaging plates. The advantage of the latter is the possibility to accumulate the signal over longer periods (up to several half-lives) and thereby to raise the sensitivity of the method. The major drawbacks of TLC are its lower resolution and the low speed of evaluation. Recent developments trying to overcome these problems are techniques like high performance TLC (HPTLC) using highly-developed TLC phases, or overpressured TLC (OPTLC) applying forced flow of the eluent onto the TLC plate which is positioned inside a hermetically sealed chamber (Witkiewicz and Bladek, 1986; Mazière et al., 1993; Morovján et al., 2002).

A further separation technique applicable in metabolite analysis is solid phase extraction (SPE). This method might be of relevance when only plasma tracer analysis or isolation of single metabolites is desired. The concept of separation is based upon a selective adsorption of either the tracer itself or selected metabolic products on small-size cartridges filled with an appropriate adsorbent which can be chosen from a wide variety of commercially available materials. Several kinds of modified silica and ion exchange resins are in use. A quite sophisticated application of SPE is described by McLellan and coworkers (McLellan et al., 1991) where a combination of anion and cation exchange columns and extraction of the effluent with alumina is utilized to separate 6-[^{18}F]fluoro-L-3,4-dihydroxyphenylalanine ([^{18}F]-FDOPA), a widely applied PET tracer, from its metabolites. One advantage of the SPE method is the possibility to handle large sample volumes when compared with other separation techniques. For plasma or urine samples usually no pre-treatment is necessary since the protein compounds of the biological matrix which cause the main problems in HPLC and TLC do not interfere with the adsorbent. However, SPE is usually not suitable to separate the whole profile of metabolites since its selectivity is low.

Special challenges arising from the analysis of radiotracers labelled with short-lived isotopes

Although the procedure of metabolite analysis of PET tracers is comparable to that of xenobiotics or drugs in general, some specific limitations arise from the low amounts of the compounds of investigation as well as from the short half-lives of the PET isotopes applied. The total quantity of a labelled compound administered during a PET examination is usually in the picomolar to nanomolar range, several orders of magnitude below pharmacological levels (Meyer et al., 1993). Therefore, the amounts of metabolites that could be separated from the samples obtained are for most applications not sufficiently high to be identified, e.g., by LC/MS. On the other hand, an example has been shown where the quantitation of a known non-metabolised tracer compound by a LC/MS method was accomplished (Forngren et al., 2000). For the reasons mentioned above, indirect methods of identification have to be applied. These could be carried out either by separate experiments in model systems with measurable amounts of the non-labelled parent compound added (also referred to as ''carrier-added'' or ''c.a.'' experiments), or by separate chemical or biochemical synthesis of the non-labelled compounds that are assumed to be the metabolites and their use as references for the analytical methods.

A further challenge might arise when, in particular, experiments with short-lived positron-emitting isotopes carbon-11 ($t_{1/2} = 20.39$ min), nitrogen-13 ($t_{1/2} = 9.97$ min), and fluorine-18 ($t_{1/2} = 109.77$ min) are carried out which require the quantitative determination of parent compound and/or metabolites by HPLC during longer study periods. In those cases, measurement with on-line activity detectors suffers from poor sensitivity. To overcome this difficulty, it is recommended to collect the fractions after separation and to quantify the amount of radioactivity off-line via measurement by high-sensitivity gamma-counters (Mazière et al., 1993; Greuter et al., 2004).

In vitro model systems used for metabolite analysis: cell systems, tissue samples, and perfused organs

Initial tests concerning metabolic liability of a compound of interest are performed using model systems such as

microsomes, cell lines, primary cells, tissue samples, including tissue sections and homogenates, or perfused organs (Sewing, 1994; Lerche-Langrand and Toutain, 2000; Houle et al., 2000; Pissarek et al., 2002; Zucco et al., 2004). Such experiments may give valuable information about metabolic processes *in vivo* provided that cells, tissues, and organs are carefully chosen and prepared. Appropriate cell types or tissue samples should contain a set of typical receptors and enzymes that represent metabolic characteristics which are comparable to the original tissue. The use of more complex model systems such as tissue-like structures and perfused organs takes into consideration that cells should preserve their functionality optimally under conditions which are adapted to their natural microenvironment (DeGrado and Moka, 1992; DeGrado et al., 2000; Lehmann et al., 2001; de Kanter et al., 2002).

Such simplified systems offer a number of advantages compared to the complex system of an entire organism. Preparation of slices and cells is relatively easy and standardization of the procedures possible. Dosing, concentration, and duration of exposure to the compound of interest can be controlled more accurately and the reproducibility of the results in general is higher since in-species variations, as weight, nutrition status, or any health effects, do not influence the tests. Moreover, comparative experiments using cells or tissues of humans and of different animals may allow conclusions about metabolic differences and similarities between species and about the suitability of animal models for further investigations (Stammati et al., 1981; Ekins et al., 1996; Olinga et al., 1997). Tissues or cells of human origin are expected to allow more reliable conclusions about the *in vivo* situation in man than investigations in animals alone; however, their use may be limited by ethical reasons. Finally, the use of cell cultures or tissue sections allows a considerable reduction in the use of experimental animals. Recently, improved cryopreservation techniques for tissue sections have been established and have demonstrated their usefulness for investigations of phase I and II metabolic activities in different kinds of tissue samples (Martignoni et al., 2004). As a result, tissue banks can make the material available on demand and improve the transparency of the followed protocols. Tissue banking can further increase the efficiency of use of rarely obtainable tissues, e.g., those of human origin.

In conclusion, metabolic studies on *in vitro* systems like cell cultures and tissue slices can be considered a valuable tool in addition to experiments on laboratory animals. Some limitations may arise from the over-simplifications made by these systems, since they lack the complexity of living organisms and the interactions of different organs. Caution is therefore advised when a proper quantification of metabolic processes is desired.

In vivo model systems used for metabolite analysis: animal models

Experiments in animal models are performed to mimic the more complex relationships between different organs and tissues in a living organism which cannot be represented by the *in vitro* models mentioned above. One prerequisite for a useful animal model is a comparative similarity of the biological property under investigation to the human conditions (Melby, 1987; Lambrecht, 1996; Sinusas, 1998). In this sense, closely related species should be the best analogues to be used in experiments but certain general metabolic pathways are shared by many species on cellular and molecular level. The species most often used in animal PET studies are rats and mice, including models of disease, since they are economical to acquire and maintain and are breed for biomedical research. They are valuable models, e.g., for initial screening experiments. For further evaluation of specific processes, the use of larger mammals such as pigs, rabbits, dogs, or monkeys is common. (Sinusas, 1998; Smith et al., 1998; Vorwieger et al., 1998; Bauer et al., 2002; Boisgard et al., 2003; Roselt et al., 2004). The animal facilities as well as all procedures which include experiments on animals will have to be approved according to the appropriate national animal welfare regulations. Metabolic investigations in animals allow, for instance, the evaluation of the normal physiological excretion ways of a compound of interest. For that purpose, urine and content of intestine can be analysed in addition to the PET scan. From the typical products identified one can conclude to the metabolic changes which the compound undergoes in the organism.

Additionally, analysis of blood samples can be done to obtain information about the derived compounds present in the circulation. To obtain detailed insight into metabolic processes in specific organs, one can choose the objects of interest from the PET images which indicate the areas of noticeable radioactivity accumulation. These organs or tissues will then be separated and subjected to a standardized pre-treatment followed by the analytical procedures.

As for the *in vitro* models, caution is advised when extrapolating the data from animal experiments to the conditions of the human body. Although the general mechanisms might be shared by the different species,

exceptions may occur and the rates of conversion may differ considerably. As an example, for rodents which are by far the most popular animal models for general metabolism investigations it is known that the rates of metabolic processes are usually accelerated notably in comparison to humans (Lambrecht, 1996). That means, e.g., that some metabolic products which are found to a greater extend in rodents may be of low relevance or may be even not present in humans during the periods of interest.

Sample preparation for metabolite analysis

Preparation of samples from in vitro tests

Experiments with cell culture systems are usually performed either on immobilized cells or in suspension cultures depending on the cell type and design of the test. The pre-treatment processes are in principal the same for both types of experimental approach. In order to detect the possible metabolic products inside the cells as well as those released into the medium, investigation of both the supernatant and the lysate of cells is necessary. Therefore the sample is first divided into liquid and solid fraction by centrifugation or simple suction of the supernatant. Since the culture medium contains several ingredients that might interfere with the analytical procedures, e.g., higher concentrations of special buffer salts or large molecules as serum proteins, the matrix has to be adapted to the experimental needs. This is usually done by precipitation of proteins with an appropriate organic solvent, e.g., methanol, ethanol or acetonitrile, or by strong acidification and, especially in case of an HPLC analysis, followed by conditioning to the eluent. Other pre-analytical methods such as SPE are also applicable for the separation of the compounds under investigation from the experimental matrix. In each case one has to test carefully which preparation is the best regarding influence on the analytes as well as need of time and recovery of radioactivity.

Of note, studies using γ-radiation for radioactivity detection provide the possibility to directly obtain total recovery of the radioactivity from all samples. This is an important advantage when compared with β^--emitting nuclides such as tritium or carbon-14 that essentially need sample solubilisation and the use of scintillation cocktails.

The isolated cell fraction is subjected to a procedure in order to release the compounds of interest and to bring them into a formulation adapted to the following analytical procedure. Break-up of the cell membranes can be achieved either enzymatically or in a hypotensive manner by addition of an excess of deionized water, whereas the latter procedure lowers the risk of an undesirable influence of the pre-treatment onto the analytes. The insoluble components are then removed by centrifugation or filtration and the remaining supernatant is analysed after removal of proteins as described above. Tissue samples from *in vitro* tests are treated according to the procedures described for the preparation of animal samples. In addition, the culture medium can be analysed after the appropriate pre-treatment.

Preparation of animal specimen

The samples commonly analysed accompanying PET examinations with animals are blood, urine and several tissues (Mazière et al., 1993). Whereas blood and urine are less critical in preparation some difficulties may arise in the extraction of the radiolabelled compounds of interest from tissue samples. Such samples require as the first step of isolation a mechanical dissection of the material which can be achieved by several methods as homogenisation by Potter-type systems, devices with cutting blades as Ultra Turrax® and Polytron® or sonification techniques. Depending on the nature of compound and the method chosen, the procedures may be performed in diverse buffer solutions, organic solvents or mixtures of both. Furthermore, pre-analytical conditions have to be optimised with respect to temperature as well as hydrolytic and oxidative side reactions. After homogenisation, the next step is the removal of those matrix components that may disturb the analytical process. These are mainly larger proteins that may be removed by precipitation or SPE techniques as described for cell cultures or by ultrafiltration procedures.

Concerning blood and urine samples, the pre-analytical procedure consists mainly of a separation step followed by protein elimination as described. The separation step aims at the removal of solid components and of red blood cells, which might be analysed separately, if necessary, after lysis and appropriate treatment as mentioned for the cell fraction in the *in vitro* tests. The analysis of the blood cells is of relevance in those cases where larger amounts of activity are retained in this compartment and hints on a specific metabolic activity are given. All pre-treatment procedures described share the need for evaluation of radioactivity recovery. If not carefully checked, one or several metabolic products might be lost in the course of sample preparation thus limiting the strength of the analysis. Special attention must be paid on compounds of higher lipophilicity since they can influence the

recovery by sticking to the test tubes or to the pellets separated. On the other hand it is important to check during the preparations that the analytes are not modified by any step of the pre-analytical procedure, e.g., by strong acidification.

Examples from food sciences

Carbon-11- and fluorine-18-labelled amino acids

Amino acids are of special importance in every living organism. They are not only building blocks of peptides, proteins and several other natural products, but some of them also act as molecular cues or are precursors of those. Moreover, amino acids are essential components of nutrition and play a role as metabolic products in the body (Koolman and Röhm, 1998). Based on their great importance for the organism numerous attempts have been undertaken to introduce several amino acids or their derivatives as PET tracers. Consequently, a great number of investigations have been performed using labelled amino acids in PET examinations striking several goals. In many cases the respective metabolic analyses were included. Resulting from the history of positron emission tomography as a diagnostic tool in nuclear medicine, most publications in this area have so far dealt with the development of labelled amino acids as tracers for tumour imaging. Established compounds to study both the local uptake of amino acids and protein synthesis which are known to be increased in malignant neoplasms are, e.g., ^{11}C-labelled L-methionine or tyrosine and ^{18}F-labelled fluorotyrosine (Ishiwata et al., 1988; Coenen et al., 1989; Vaalburg et al., 1992; Pruim et al., 1995; Willemsen et al., 1995; Jager et al., 2001) (Fig. 1). Other tracers target the local amino acid uptake alone but are not incorporated into proteins, e.g., O-fluoromethyl-, O-fluoroethyl- and fluoro-α-methyl tyrosine (Jager et al., 2001; Laverman et al., 2002; Ishiwata et al., 2004).

Several methods have been described for the analysis of blood metabolites of the above mentioned amino acids, e.g., membrane filtering techniques with subsequent RP-HPLC separation of the low-molecular fraction (Lundquist et al., 1985), or simple HPLC after separation from the protein-bound fraction (Ishiwata et al., 1988; Ishiwata et al., 1989). For 1-[^{11}C]- or 1-[^{14}C]-labelled amino acids, the formation of labelled CO_2 was assessed in several cases by acidification of the appropriate samples as described by Willemsen and colleagues (Willemsen et al., 1995). Incorporation of the amino acids under investigation into peptides and proteins was evaluated by measurement of radioactivity in supernatant and pellet after acidic deproteinization, e.g., by trichloroacetic acid, or by gel filtration chromatography to assess the incorporation into proteins (Ishiwata et al., 1996; Lundquist et al., 1985). Most of the tracers mentioned above have been developed for PET investigations highlighting processes in the brain, in particular brain tumours, therefore the metabolic analyses were mainly focused on plasma tracer analysis as a prerequisite for the quantification of the images.

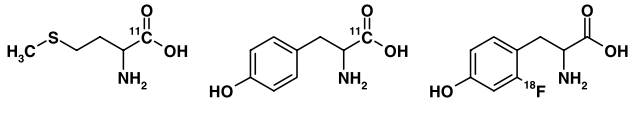

Fig. 1. Structures of methionine and tyrosine based tracers established for measurement of protein synthesis by PET

A further labelled amino acid that has gained great attention as a tracer of presynaptic dopaminergic function in human brain is 6-[^{18}F]fluoro-L-3,4-dihydroxyphenylalanine, also referred to as FDOPA. Extensive metabolic examinations of FDOPA have been described revealing a number of labelled products, namely 3-O-methyl-6-[^{18}F]fluoro-L-DOPA (3-OMFD) and 6-[^{18}F]fluorodopamine, as well as a number of products resulting thereof, such as fluorohomovanillic acid and dihydroxy fluorophenylacetic acid, were found in brain, plasma and other peripheral organs (Firnau et al., 1988; Melega et al., 1990, 1991; Miletich et al., 1993; Pauwels et al., 1994; Ruottinen et al., 1997; Vorwieger et al., 1998; DeJesus et al., 2000; Endres et al., 2004).

The analytical procedures applied usually consisted of an acidic protein precipitation followed by RP-HPLC separation, in several cases under ion-pair conditions. Identification of the metabolic products was achieved by comparison of their retention time with those of authentic non-radioactive standards (Luxen and Barrio, 1988; Luxen et al., 1993; Vorwieger et al., 1998). A simpler but quite sophisticated method was described using ion exchange cartridges followed by alumina extraction to separate 3-OMFD and FDOPA from the rest of the products (McLellan, 1991). This procedure allows a higher sample throughput compared to the LC method and represents a satisfactory solution for the creation of an input function for PET examinations although it is not able to separate all of the labelled products.

More recently some experiments in model systems *in vivo* and *in vitro* have been carried out using amino acids and peptides labelled by [^{18}F]fluorobenzoylation of amino groups (Wester et al., 1996; Bergmann et al.,

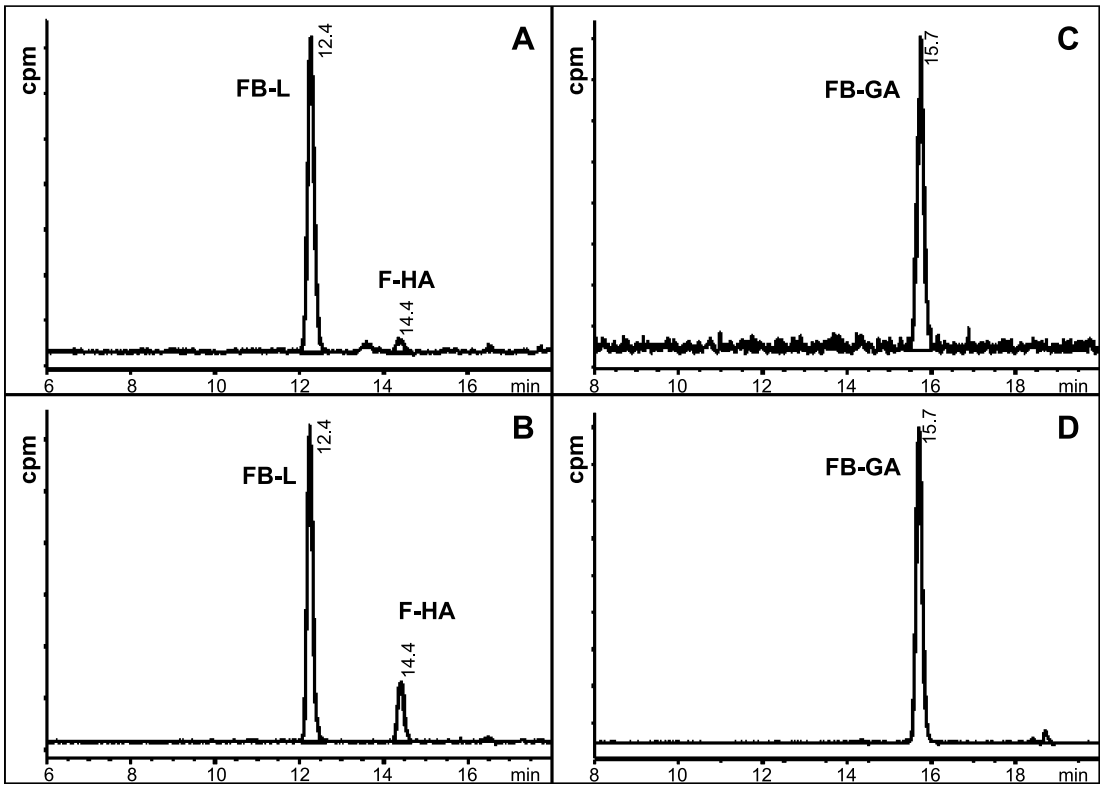

Fig. 2. RP-HPLC of plasma (top) and urine (bottom) of rats after *i.v.* administration of **FB-L** (60 min *p.i.*, A + B) and **FB-GA** (45 min *p.i.*, C + D); FB-L N^{α}-(4-[^{18}F]fluorobenzoyl)-lysine, FB-GA N-(4-[^{18}F]fluorobenzoyl) glutamic acid, FB-HA 4-[^{18}F]fluorohippuric acid

2001; Wuest et al., 2003). In particular, these investigations have dealt with modified amino acids and isopeptides that are of specific relevance in food sciences (Bergmann et al., 2001; Wuest et al., 2003). The experiments were prompted by a study on the metabolism of the [^{18}F]-labelled isopeptide N^{ε}-(γ-glutamyl)-L-lysine (**FB-GL**) in rats which is reported elsewhere in this issue in more detail (Hultsch et al., 2005). In this context, N^{α}-labelled lysine (**FB-L**) and N^{α}-labelled glutamic acid (**FB-GA**) were investigated as potential metabolites of **FB-GL** assuming a cleavage of the isopeptide bond *in vivo*. The subsequent metabolism of these compounds was assumed as part of the **FB-GL** degradation. As a result, the analysis of **FB-GA** did not show any abundant metabolite in blood or urine, whereas **FB-L** showed the formation of [^{18}F]fluorohippuric acid (**FB-HA**, present in plasma, urine, and kidneys) which represents the conjugation product of [^{18}F]fluorobenzoic acid with glycine (Fig. 2). These examinations were done by means of RP-HPLC after acidic deproteinization and correlated well with TLC analyses. Identification of the metabolite as [^{18}F]fluorohippuric acid was achieved by comparison of the retention time observed with that of the authentic compound. This observation, by the way, highlights another challenge when following the metabolic fate of any compound of interest via radioactive labelling which is a sufficient stability of the label itself. Obviously, the liability of N^{α}-fluorobenzoylated lysine to an exchange of the amino acid by glycine is considerably higher than that of fluorobenzoylated glutamic acid.

Fluorine-18-labelled polyphenols

As an example, resveratrol (3,4′,5-trihydroxy-*trans*-stilbene), is well known as a naturally occurring polyphenolic compound which is found, e.g., in grapes and several medicinal plants and has been attributed to be of relevance for human nutrition for a long time. Recently, a variety of advantageous biological properties have been ascribed to resveratrol, including antifungal, antibacterial, antiviral, anticancerogenic, as well as cardioprotective effects (Frémont, 2000; Middleton et al., 2000; Wu et al., 2001; Savouret and Quesne, 2002). Quite a large number of investigations have dealt with the metabolism and distribution of resveratrol. However, non-invasive PET studies are a new field of this research. Very recently, synthesis and biological evaluation of a novel ^{18}F-labelled resveratrol derivative have been performed by Gester and

colleagues and are described in this issue in more detail (Gester et al., 2005). For this new tracer compound, 3,5-dihydroxy-4′-[^{18}F]fluoro-*trans*-stilbene (**F-RV**), an accompanying metabolic analysis in the rat was done in plasma, urine, and content of duodenum, where higher amounts of the radioactivity administered had been detected during the PET scan. Additionally, fractions of cellular uptake experiments using a human hepatoma cell line (HepG2) were investigated. Analysis was done by RP-HPLC after ethanolic precipitation of proteins. The observations in animal experiments revealed a fast and nearly complete conversion of the parent compound resulting in two main metabolites (Mb1 and Mb2), which were present in plasma and urine (Fig. 3b, c) as well as in the content of duodenum. Furthermore, one of these conversion products (Mb1) was also detected in cell culture supernatants after incubation with hepatoma cell line HepG2 (Fig. 3a). From data reported by others on the metabolism of resveratrol in mice, rats, and human, it is assumed that these metabolites Mb1 and Mb2, considering the substitution of the 4′-OH group for ^{18}F, are 3-*O*-glucuronides and -sulfates of **F-RV** (Kuhnle et al., 2000; de Santi et al., 2000a, b, c; Yu et al., 2002; Goldberg et al., 2003; Vitrac et al., 2003; Walle et al., 2004; Gester et al., 2005).

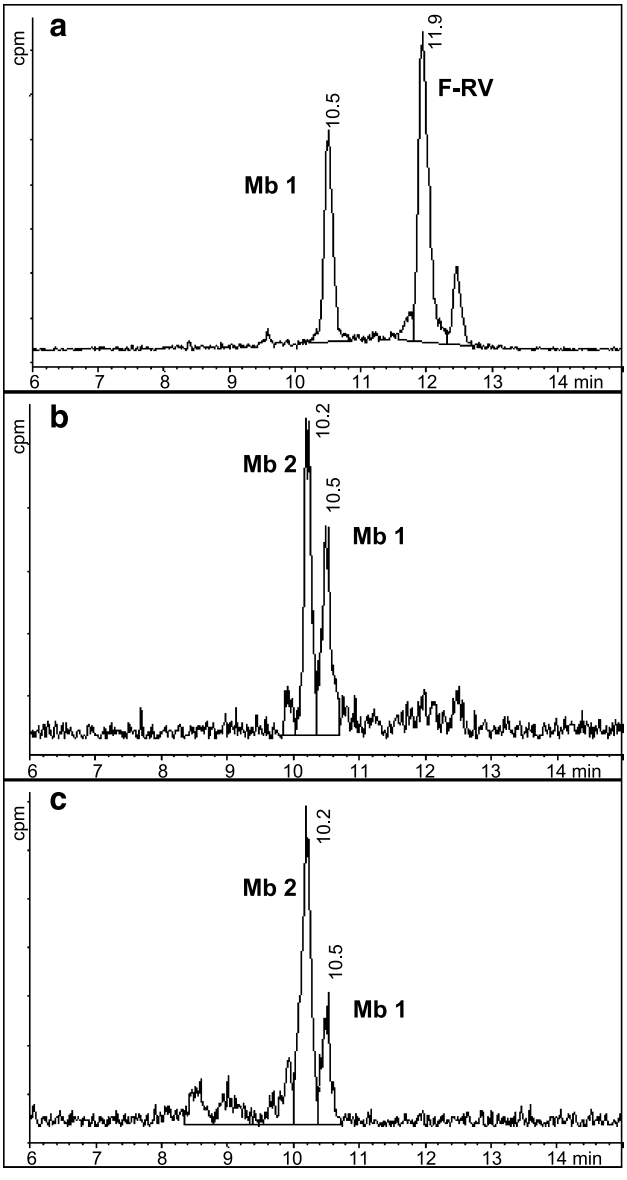

Fig. 3. RP-HPLC of (top) supernatant of cell culture after incubation with **F-RV** (30 min, 37°C, HepG2 cell line), (middle) plasma 5 min *p.i.* and (bottom) urine 55 min *p.i.* of a rat after *i.v.* administration of **F-RV**

Since the experiments *in vivo* were performed after intravenous administration of the labelled compound, the practical relevance of the results obtained for nutritional considerations is limited. It was reported that the bioavailability of resveratrol after oral administration is very low (Goldberg et al., 2003; Walle et al., 2004). In order to gain deeper insights into physiological processes which are of a higher relevance for food sciences, e.g., bioavailability or digestive transformations, additional examinations under more physiological conditions, e.g., after oral administration of the tracer, would be desirable.

Fluorine-18-labelled AGE model compounds

Advanced glycation endproducts (AGEs) resulting from the *Maillard* reaction between reducing carbohydrates and peptides or proteins are reported to be of interest from the nutritional as well as the physiological and pathophysiological point of view (Singh et al., 2001; Henle and Miyata, 2003; DeGroot, 2004). For the study of the *in vivo* behaviour of two representative AGEs, N^{ε}-carboxymethyllysine (CML) and N^{ε}-carboxyethyllysine (CEL), respectively, in rats PET was applied after labelling of the compounds by [^{18}F]- fluorobenzoylation (Bergmann et al., 2001). The fluorobenzoylated AGEs are considered a model for dipeptides modified by *Maillard* reaction which might be released during digestion. In the PET studies, mainly renal excretion of the radioactivity was observed in connection with missing evidence for enterohepatic circulation of the labelled compounds. It was concluded that metabolites, if any, should be detectable in urine. Analysis was therefore, besides plasma samples, performed in the urine. In addition, plasma samples from *in vitro* incubation experiments in whole blood were investigated. The analytical procedure was accomplished

without prior deproteinization by on-line coupling of SEC and RP-HPLC and additionally by TLC on RP sheets. It was reported that neither fluorobenzoylated CML nor CEL showed any remarkable metabolisation in plasma or urine and that none of the compounds seemed to be utilized for protein biosynthesis (Bergmann et al., 2001).

Conclusion

Metabolite analysis in terms of identification of metabolic pathways of a compound of interest as well as quantification of their metabolic products for a given period of time has been shown to be a prerequisite for quantitative PET investigations and moreover to be of interest for a deeper understanding of physiological as well as pathological processes in the body. Some specific challenges arising from the PET methodology, namely the short half-lives of the nuclides utilized as labels as well as the trace amounts of drugs to be analysed require some specific analytical techniques. HPLC analysis with on-line radioactivity detection (radio-HPLC) seems to be the most widely applied method for routine determination of metabolites, since it is sensitive, easy to automate and compatible with several modes of additional detection. For higher sample throughput in routine operation, OPTLC or TLC methods, which allow simultaneous processing of large sample numbers are valuable alternatives. However, especially the latter one suffers from poor resolution. For all chromatographic techniques applied, pre-analytical procedures aimed at the extraction of the compounds of interest from the matrix and adaptation to the individual needs of the analytical method are of special importance. Special attention has to be paid to assure that the compounds of interest are resistant against the conditions applied, e.g., reactants, extreme pH or temperature. In cases where quantification of the metabolic products and/or the parent compound is intended, a careful evaluation of the recovery of each of them is necessary. In this regard, incomplete extraction of one or more compounds as well as adsorption effects or non-specific binding to proteins might be sources of errors and could be easily detected by determination of activity distribution in every step of the treatment.

The application of model systems for investigations on metabolisation of a radiotracer is common practice whereas the choice of the model strongly depends on the intention of the experiment. The relevance of the results obtained in cells, tissues or laboratory animals for the human organism must be considered carefully. As well, not only the nature of the model but also the mode of application of a compound of interest should be discussed critically.

Although PET measurements can be performed after any route of drug administration, intravenous, inhalation or oral, by far, intravenously administered drugs have been the most extensively studied. Studies of inhaled or orally administered drugs are clearly of great interest; however, formulation issues have precluded widespread applications in these areas. Despite these difficulties, Berridge and coworkers (Berridge et al., 1998; Berridge and Heald, 1999) performed an elegant series of studies with [^{11}C]-triamcinolone acetonide delivered by inhalation. In these studies, the tracer was introduced into a standard drug inhaler system, thoroughly mixed with unlabeled drug and administered by the same method that is used for the unlabeled drug. However, in examinations with respect to food sciences an oral application of the labelled substances should be considered. The inclusion of gastrointestinal processes which may contribute to a wide extent to the metabolism of a compound should raise the physiological relevance of the experiments although in several cases the relative short half-lives of the radioisotopes usually applied in PET compared to the rate of digestive processes might limit the applicability of this approach. Furthermore, in the case of orally administered drugs formulation issues are even more formidable. Although radiolabelled drugs can be easily swallowed, the time course of drug absorption from the gastrointestinal tract into the circulation is highly dependent on thorough mixing in formulating pills or capsules. Since it is almost impossible to achieve the same formulation used by drug manufacturers within the time-frame of the short half-lives of PET radionuclides, these types of studies are extremely difficult to perform. Recently, first PET studies of tissue pharmacokinetics of orally administered drugs have been reported (Fischman et al., 2000).

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