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Review Article

^{18}F-labeled fluorodeoxyglucose for PET imaging: the working mechanism and its clinical implication

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Introduction

Positron emission tomography (PET) is a sophisticated imaging technique to assess functional abnormalities in clinical practice. PET uses radioactive tracers that are labeled with radionuclides such as ^{11}C, ^{13}N, ^{15}O and ^{18}F. These radionuclides (positrons) offer the unique possibility that they can be chemically incorporated in compounds that can be used to investigate metabolically important processes. With PET it is possible to obtain tomographic images, which are reconstructed from the detection of the simultaneous emission of two back-to-back 511 keV photons emitted by the positron labeled molecule. In a PET camera this detection occurs with a detector ring in which scintillation detectors are configured to form a fixed ring around the patient.

PET has opened a new field of diagnostic imaging as it allows the measurement of tissue metabolism *in vivo*. As such it is essentially different from other imaging modalities which provide anatomic information such as plain x-ray, computed tomography (CT) and magnetic resonance imaging (MRI). Clinical applications for PET are numerous including viability testing of the heart muscle and imaging to study neuroreceptors. The application that has most rapidly increased, however, is in the field of oncology where it is used in diagnosis and staging, in measuring response to treatment and in the detection of tumor recurrence. In this respect, the most commonly used radiopharmaceutical is ^{18}F-labeled fluorodeoxyglucose (^{18}F-FDG). This radiotracer behaves as if it were glucose, thereby reflecting cellular energetics.

As glycolytic processes are upregulated in cancer, the higher accumulation of FDG in malignant lesions makes it possible to detect and evaluate tumor sites and tumor metabolism with PET. However, several factors, such as serum glucose levels, tumor hypoxia and malignancy grade, may influence the uptake at these sites. In addition, iatrogenic and therapeutic changes may interfere with one's ability to make a reliable judgement of the primary or recurrent tumor status. Therefore, knowledge of the basic aspects of FDG uptake is a prerequisite for an optimal interpretation of FDG PET studies.

This article summarizes the current knowledge of the uptake mechanism and biochemical processing of glucose and FDG in normal and malignant cells. Some factors that influence uptake of FDG and the implication for clinical studies are also discussed.

Uptake of glucose by normal cells

Glucose is the most important supplier for the synthesis of tissue, and energy-producing metabolic processes depend largely on glucose availability.

The uptake of glucose and other hexoses by human cells can take place via three mechanisms of transport. The first, passive diffusion, is a relatively slow process and is made possible by the hydrophilic nature of glucose. It is of minor importance for human tissue. The second mechanism is active transport by a Na^+-dependent glucose transporter, occurring across the apical brush border of intestinal and kidney epithelial cells. The third method of transport involves the facilitative glucose transporter (GLUT), which is present on membranes of almost all cells and is the main pathway for glucose to enter the cell body.

Na^+-dependent glucose transporter

Sodium ion is a cofactor for the active transport of glucose across intestinal and kidney epithelial cells. This process is facilitated by sodium-glucose transporters (SGLT) of which two different isoforms are known. SGLT-1 has a high affinity for glucose and transports Na^+ and glucose in a 2:1 ratio, whereas SGLT-2 has a low affinity and the Na^+ to glucose ratio is 1:1 (1). Another difference

between SGLT-2 is that SGLT-1 can transport glucose as well as galactose (2).

Glucose transporters

At least five active glucose transporters have been identified and share the same cellular transmembrane topology. However, their kinetic properties, tissue localization and mechanism to regulate glucose homeostasis differ. Although GLUT-6 and GLUT-7 have been biochemically recognized, their specific function has not been elucidated (3, 4).

GLUT-1

This transporter is widely expressed at membranes of many different cells in the human body; the highest concentrations are found in fetal tissue (5) and placenta (6). Thus, it is not very surprising that growth stimuli, like insulin (7), insulin-like growth factor-I (8), growth hormone (9) and thyroid hormone (10), induce a higher expression of GLUT-1.

In adults, high expression is seen in erythrocytes (11) and in epithelial cells of blood-tissue barriers, especially the blood-brain barrier (12). Besides glucose, GLUT-1 can also transport galactose and mannose. This takes place in an asymmetrical way, *i.e.*, the affinity for sugar influx is about 10 times as high as glucose efflux (13).

It has been suggested that GLUT-1 is part of the family of the glucose regulated proteins, of which the genes are more expressed in situations of cellular stress (14).

GLUT-2

This low-affinity transporter can be found in intestine, kidney, liver and the β-cells of the pancreas (15). It is also present in different regions of the brain (16, 17). Together with the glycolytic enzyme glucokinase, it constitutes a glucose-sensing system, which signals the differences in glycemia to the liver and the pancreatic β-cells (18). Glucose, galactose, mannose and fructose can all be carried by the GLUT-2 transporter.

GLUT-3

Due to its high affinity for glucose, GLUT-3 can assure a constant glucose supply to neurons in the brain, even at low extracellular glucose concentrations (19). In the brain, glucose is transported across the blood-brain barrier by GLUT-1 and once it reaches the neurons, GLUT-3 is the most efficient transporter in the hypoglycemic conditions of the cerebral interstitial space. GLUT-3 has a high expression in the testis as well, especially in spermatozoa (20).

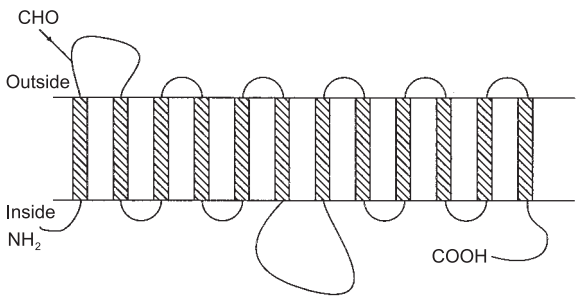

Fig. 1. Orientation of glucose transporter protein in the plasma membrane (from Kasanicki and Pilch, ref. 81).

GLUT-4

Expression of this transporter is high in brown and white adipose tissue and in skeletal and cardiac muscle. In hyperglycemic circumstances like the postprandial period (period after dinner), glucose transport can be increased up to 30 times in response to insulin. Insulin stimulates intracellular vesicles, which contain the GLUT-4 transporter, to translocate to and fuse with the plasma membrane. The thus increased GLUT-4 expression results in an increased glucose uptake of the cell (21, 22).

GLUT-5

This transporter is only 39-40% identical to the other isoforms (3). It is the main transporter of fructose and is expressed at high levels in the jejunal region of the small intestine, although its mRNA can also be found in small concentrations in kidney, brain, insulin-sensitive tissues, testicles and spermatozoa (23). There is some evidence that glucose is a competitive inhibitor of fructose uptake at GLUT-5. This differential sensitivity of fructose to glucose may be due to proteins interacting with GLUT-5, as suggested by Miyamoto *et al.* (24).

Molecular structure and function of GLUT protein

In the GLUT molecule, hydrophilic loops connect a structure of 12 membrane domains; both the N terminus and the C terminus are on the cytoplasmic side (Fig. 1).

An alternating conformer model is used to describe the transport of glucose by the GLUT protein across the cell membrane (Fig. 2). The transporter has one sugar binding site that can be exposed both at the cytoplasmic surface and the extracellular surface but not simultaneously at both. Sugar binds to the extracellular surface and a carrier-sugar complex is formed. This carrier-sugar complex can reorientate to the opposite side of the membrane by virtue of a conformational change and, finally, sugar is released into the cell (25). The cytoplasmic side of divergent amino acid sequence seems to be important

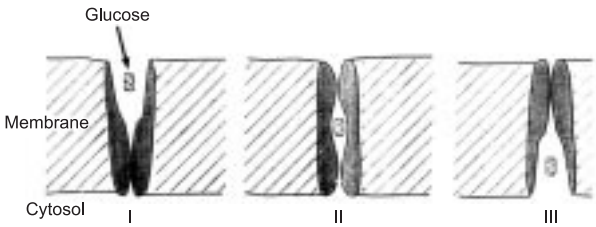

Fig. 2. Transport of glucose molecule across the cell membrane facilated by the glucose transporter protein.

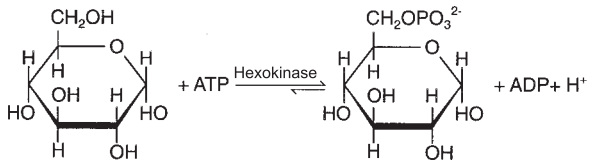

Fig. 3. Conversion of glucose to glucose-6-phosphate, catalyzed by the enzyme hexokinase.

in the regulation of GLUT. Jung *et al.* (26) hypothesized a role for a specific cellular protein, binding via a certain pathway to the cytoplasmic side. This, as yet, unidentified protein seems to be of major importance in the insulin-induced GLUT recruitment.

Fate of glucose in normal cells

As already mentioned, glucose is the main building block for the synthesis of tissue and the generation of energy. To fulfil this role, glucose undergoes glycolysis, which is the first metabolic step in the process to convert glucose to pyruvate and lactate.

Glycolysis

This is a set of multiple reactions, occurring in the cytosol, eventually converting one glucose molecule into two molecules of pyruvate. Glycolysis has three control elements represented by irreversible reactions, having regulatory as well as catalytic roles. The first reaction is such a control element, the conversion of glucose to glucose-6-phosphate (Fig. 3). This reaction is catalyzed by the enzyme hexokinase, which binds to the outer membrane of the mitochondrion near the porin molecule. These porins represent voltage-dependent anion channel isoforms (27) and they are protein channels, through which ATP moves outside the mitochondrion to enter the cytosol. Hexokinase reduces the ATP to ADP and produces glucose-6-phosphate. Meanwhile, ADP enters the mitochondrion again and acts as an acceptor for phosphoryl groups. The porin-hexokinase complex is stimulated by insulin.

So far four different types of hexokinase are known. This division is based on their electrophoretic mobility. Type I is most abundant in brain and erythrocytes, type II is predominant in insulin-sensitive tissue, such as skeletal muscle, heart, diaphragm and adipose tissue, type III is not predominant in any tissue (28) and type IV, also called glucokinase, is predominant in liver cells.

The enzymatic activity of hexokinase is inhibited by glucose-6-phosphate, except that of glucokinase. When glucose is abundant, glucokinase becomes activated in the liver to produce glucose-6-phosphate for the synthesis of glycogen.

Another important step is the conversion of fructose-6-phosphate to fructose-1,6-biphosphate (Fig. 4). This reaction is catalyzed by phosphofructokinase, which is the most important control element in glycolysis. Unlike the other control elements hexokinase and pyruvate kinase, this is the only unique irreversible step in glycolysis (*i.e.*, it catalyzes the reaction of molecules that are strictly glycolytic intermediates). Phosphofructokinase is allosterically inhibited by ATP, inhibiting glycolysis when there is enough energy, and by H^+ (preventing excessive formation of lactate). Another inhibitor is citrate, which is an intermediate of the citric acid cycle (see below) and abundant when there are high levels of biosynthetic precursors. A remarkable activator of phosphofructokinase is fructose-2,6-biphosphate, which is formed out of fructose-6-phosphate by an enzyme called phosphofructokinase 2. When fructose -6-phosphate is abundant, this system will lead to a rise in fructose-2,6-biphosphate levels and hence to stimulation of phosphofructokinase.

The third irreversible step in glycolysis is the reaction phosphoenolpyruvate-pyruvate catalyzed by pyruvate kinase (Fig. 5). Three types of pyruvate kinase have been

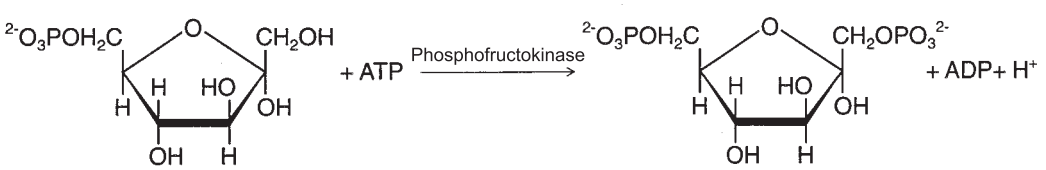

Fig. 4. Conversion of fructose-6-phosphate to fructose-1,6-biphosphate, catalyzed by the enzyme phosphofructokinase.

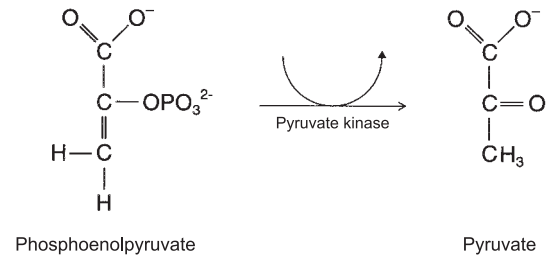

Fig. 5. Conversion of phosphoenolpyruvate to pyruvate, catalyzed by the enzyme pyruvate kinase.

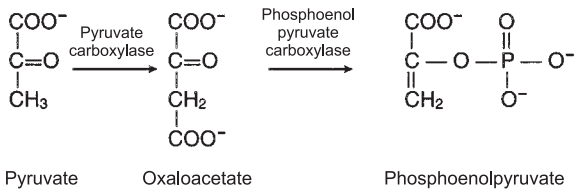

Fig. 6. Carboxylation of pyruvate into oxaloacetate, followed by the conversion of oxaloacetate into phosphoenolpyruvate.

found: L-type is predominant in the liver, M-type is predominant in muscle and brain and A-type is abundant in other tissues. ATP allosterically inhibits the isoenzyme (so glycolysis is slow when there is enough energy). Alanine, synthesized from pyruvate, is also an inhibitor, thus signaling that there are enough building blocks. The enzyme is stimulated by fructose-1,6-biphosphate.

At the end of glycolysis, pyruvate is subject to four different metabolic fates. Under anaerobic conditions, for example in muscle during intense activity, pyruvate is reversibly converted into lactate. This reaction serves to regenerate NAD^+, which is necessary for glycolysis to proceed. The overall reaction in the conversion of glucose in lactate delivers two ATP molecules, the maximum amount of energy under anaerobic conditions. A second fate of pyruvate is transamination into the amino acids alanine, valine and leucine, from which the second and third are essential. This occurs in the cytosol. These reactions are also reversible, resulting in the possibility for the amino acids to enter the central metabolic pathway and be oxidized in the citric acid cycle to generate energy. Further, pyruvate can be carboxylated inside mitochondria into oxaloacetate, an intermediate of the citric acid cycle (Fig. 6). This carboxylation serves two goals: one is replenishing intermediates of the citric acid cycle and the other is to make possible gluconeogenesis (the synthesis of glucose from noncarbohydrate sources, such as lactate, amino acids and glycerol). It shares a number of reactions with glycolysis but also includes a number of other reactions to bypass the essential irreversibility of the corresponding reactions in glycolysis. The conversion pyruvate-oxaloacetate and the following conversion of oxaloacetate into phosphoenolpyruvate is such a bypass (Fig. 6). A fourth, very important, fate of pyruvate is its conversion into acetyl-CoA inside mitochondria. This molecule can be the supplier of 2-carbon fragments needed for the synthesis of lipids or it can enter the citric acid cycle if there is a need for ATP.

The citric acid cycle

The citric acid cycle (also called the TCA cycle or Krebs cycle) (Fig. 7) occurs in the mitochondria and is the final common pathway for the oxidation of glucose and other fuel molecules. Some intermediates can also be transaminated into amino acids.

The first step in the cycle is the condensing of acetyl-CoA with oxaloacetate, thus generating a 6-carbon molecule, citrate. In the following steps citrate is again converted into oxaloacetate. Thus, two carbon atoms from acetyl-CoA enter the cycle and leave the cycle as two CO_2 molecules. The energy yield from the several steps is 3 NADH, 1 FADH and 1 GTP per molecule pyruvate. The NADH and FADH molecules transfer their electrons to oxygen in the electron transport chain to generate 3 ATP per NADH and 2 ATP per FADH. This is necessary for the continuity of the cycle, since a constant supply of the electron acceptors NAD^+ and FAD^+ is required for the cycle to proceed. Hence, the citric acid cycle is obligatorily aerobic.

The rate of the cycle depends on the need for ATP. There are several control elements in it, which are dependent on the amount of ATP: citrate synthetase, isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase. Another important control element for the cycle is the irreversible conversion of pyruvate to acetyl-CoA. As mentioned above, some intermediates can be converted into amino acids. Oxaloacetate can be transaminated into aspartate, a precursor for several other amino acids, most of them being essential. Glutamate, again a precursor for amino acids, is the product 2-oxoglutarate.

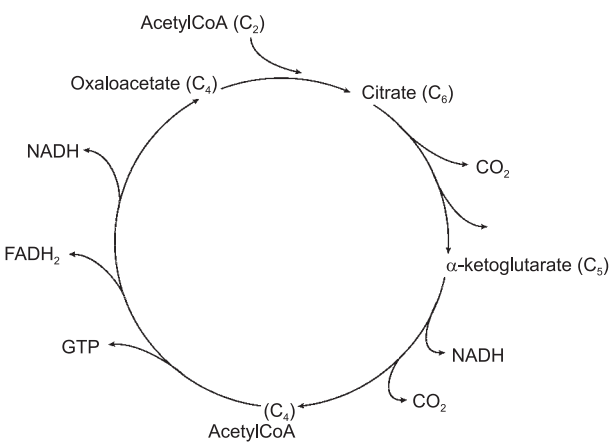

Fig. 7. The citric acid cycle.

Glucose uptake in tumor cells

Oncogenes and growth factors regulate the expression and activity of the facilitative glucose transporters. Merrall *et al.* (29) demonstrated that the overexpression of GLUT-1 and GLUT-3 in tumor cells is the main cause of enhanced glucose transport. Research performed in the nineties revealed the following: an increase in GLUT-1 and GLUT-3 mRNA in cancers of esophagus, colon (together with GLUT-5 (30)) and pancreas (31); overexpression of GLUT-1 and GLUT-3 mRNA in the brain (32) and together with GLUT-1 protein expression in head and neck tumors (33); GLUT-1 overexpression in renal cell carcinoma (34), insulinomas (35), hemangioblastomas (36) and thyroid cancers (37); GLUT-1 and GLUT-3 overexpression in lung carcinomas (38); GLUT-1 and GLUT-5 overexpression in breast tumors (39, 40).

Younes *et al.* (41) showed that, with some rare exceptions, GLUT-1 shows limited expression in normal human tissue and benign lesions. They confirmed the work of Nelson *et al.* (42) by showing that GLUT-1 overexpression in cancer cells is not characteristic of all tumors. Furthermore, GLUT-1 overexpression varied in positive tumors from a few cells to the majority of tumor cells. The overexpression of GLUT-1 and GLUT-3 was only localized in the perinecrotic zones of the tumor, where the environment of the tumor cells is hypoxic. Abundance of glucose transporters could be an adaptation of the tumor cell to survive and represent an advantage over normal cells under such circumstances (33).

Differences in GLUT expression between tumor cells and normal tissue and the heterogeneity of GLUT-1 expression in the different tumors suggest that such expression may be of biological significance. Correlation was seen between expression of GLUT-1 and the aggressiveness of the tumor (41). For example, GLUT-1 expression in colon cancer and the frequency of lymph node metastases were found to be correlated (41).

Of clinical importance is the question whether these changes in expression of facilitative transporters are strictly related to the malignant phenotype or occur already at an early stage in carcinogenesis. Reisser *et al.* (43) found that preneoplastic lesions already showed an increased expression of GLUT-1, so increases in GLUT-1 expression might be used as a diagnostic tool for preneoplastic lesions. In the same work, they found a relationship between reduction of glycogen storage and the degree of dysplasia that was associated with increased GLUT expression.

New therapeutic strategies based on GLUT-overexpression could be developed in the future. Inhibition of GLUT-1 with chemicals such as phloretin, a known inhibitor of glucose membrane transport, could be useful (44). Other therapeutic approaches could be the competitive inhibition of glucose uptake by glucose analogs such as 3-*O*-methyl-D-glucose (45) or intracellular introduction of glucose-linked cytotoxic molecules using the transport mechanism (46).

Hexokinase in tumor cells

A very important metabolic difference between a normal and malignant cell is the fact that the latter depends to a large extent on glycolysis, whereas the citric acid cycle shows only minor activity (47). Besides production of energy, glycolytic intermediates are also involved in two other features of growing tumors: enhanced DNA/RNA synthesis by means of the pentose phosphate pathway and the synthesis of lipids (out of fructose-1,6-biphosphate), essential for membrane synthesis. The glycolytic capacity of a given tumor is now generally assumed to be characteristic for its state of differentiation (48, 49).

Singh (50) suggested that the promoting force for this increased glycolytic flux is the phosphorylation of glucose, the first reaction of the glycolytic pathway. This step is catalyzed by hexokinase and it is this enzyme that is showing an increased activity in tumor cells (28, 51). At least two factors contribute to this increased activity of hexokinase. One is the difference in binding to the outer membrane of the mitochondria, the other is the overproduction of this enzyme in malignant cells compared to normal cells.

Hexokinase binding to mitochondria

The percentage of membrane bound hexokinase increases with the grade of malignancy. Investigations by Rempel *et al.* (28) revealed that this binding percentage is about 30% in normal liver cells, while it amounts to about 70% in dedifferentiated tumor cells. Hexokinase binds to the porin molecule on the outer mitochondrial membrane and thus has easy access to ATP produced in the mitochondrion (51). The N-terminal half of hexokinase is thought to be involved in this connection with the mitochondrial membrane, while the C-terminal half has both catalytic and regulatory roles (52). The bound hexokinase is less sensitive to the feedback inhibition of glucose-6-phosphate, which is an important mechanism in normal cells (53). Also proteolytic degradation is of less influence on bound hexokinase.

It is intriguing to know that the soluble and the bound enzyme are chemically identical. Hexokinase can be resolved in two subtypes (IIa and IIb) by hydrophobic interaction chromatography. Due to its greater hydrophobicity and lower negative charge, type IIb, compared to type IIa, shows an increased binding to mitochondria. There is evidence that regression in differentiation is accompanied by alterations in portion and subcellular distribution of these subtypes (28).

Hexokinase overproduction

The four different isoenzymes of hexokinase present with a pronounced tissue specific distribution. In malignant cells, type II hexokinase and to a lesser extent

type I hexokinase are overexpressed, regardless of whether or not the respective tissue of origin expresses these enzymes (28, 54). These two isoenzymes are exactly the same as in normal tissue and in their experiments no evidence was found for a specific tumor hexokinase. This does not exclude a posttranslational modification, regulation by phosphorylation for example, seen already *in vitro* but not yet *in vivo* (55, 56).

Increased transcription of the hexokinase gene seems to be, at least in part, the cause for the overproduction of the enzyme in cancer, as a marked elevation of hexokinase mRNA levels has been demonstrated in tumor cells (57). This was demonstrated by Mathupala *et al.* (54) who found a 10-fold transcriptional rate of hexokinase in tumor cells.

Research has been done on the promotor region of type II hexokinase to uncover the transcriptional regulation of the overexpression in tumor cells. The promotor binding site for RNA polymerase on a gene in the process of RNA synthesis contains various response elements. These are sequences via which several transcription factors can exert their regulatory influence on expression of the enzyme.

Hexokinase production and insulin and glucagon

In normal cells, insulin is active in the fed state and glucagon in the fasted state. Therefore opposing effects on gene expression were found: insulin activates the transcription of hexokinase and glucagon inhibits this transcription. This is certainly the case for type IV hexokinase (glucokinase), which is the predominant isoform expressed in normal liver, where the type II isoform is silent (58). cAMP, the major mediator of glucagon signaling, is also an inhibitor of glucokinase (59). Both insulin and glucagon (cAMP) activate the promotor of the hexokinase II gene in cancer cells. This can be seen as a strategy of the cancer cell to keep its glycolysis high, even with a limited supply of glucose. In case the glucose level in the environment rises, the cancer cell can utilize the available glucose immediately, without a lag period. This means that transcription of hexokinase in tumor cells is independent from the metabolic state of neighboring healthy cells. Thus, cancer cells have an advantage over their cell of origin.

Hexokinase and hypoxia

Wang and Semenza (60) determined the presence of a specific region inducing hypoxia in the promotor of hexokinase. The hypoxic state of a solid tumor may cause upregulation of hexokinase, which enhances the rate of glycolysis, serving as a mechanism for the tumor cell to survive. Interestingly, the response element for glucose was found to overlap the response element for hypoxia. It is not yet known whether these two elements act synergistically in the presence of both glucose and hypoxia.

Gene amplification

The overexpression of type II hexokinase in malignant cells is, besides the above described differential transcriptional regulation of the hexokinase expression, the result of another mechanism. Rempel *et al.* (61) found that in the rapidly growing rat AS-30D hepatoma cell lines, increased hexokinase activity was partly the result of an at least 5-fold amplification of the type II hexokinase gene compared to normal hepatocytes. This is not very surprising as instability of the genome is often seen in transformed cells and overexpression of oncogenes is often caused by amplification.

p53

In normal cells, p53 acts as a cell cycle checkpoint protein, thus halting the cell in G_1 phase in case of DNA damage. Mathupala *et al.* (62) discovered the presence of functional p53 response elements on the type II hexokinase promotor in another experiment with AS-30D hepatomas.

In this experiment, there appeared to be a positive regulatory effect of mutated p53 (two point mutations in its cDNA were exhibited) on the hexokinase promotor. In the AS-30D hepatoma cells, p53 was highly abundant in its mutated form, with a longer half-life than wild-type p53 (63). This could be the key to a possible link between loss of cell cycle control in rapidly growing tumor cells and their propensity to catabolize glucose at high rates.

^{18}F-labeled fluorodeoxyglucose

^{18}F-labeled fluorodeoxyglucose (FDG) is the most commonly used PET radiopharmaceutical. The half-life of the radionuclide label is nearly 2 h, which allows its transportation to distant sites where there is no cyclotron necessary for the production of the isotope. Numerous papers have appeared on the clinical use of this agent but relatively few on the mechanism of accumulation. Therefore, some features of this interesting molecule are highlighted in the following.

The glucose analog deoxyglucose, which is missing an oxygen atom compared to the glucose molecule (Fig. 8), is transported into the cell in the same way as glucose. Once it has reached the cytosol, it is phosphorylated to deoxyglucose-6-phosphate by the enzyme hexokinase, just like normal glucose. The next reaction in glycolysis to deoxyfructose-6-phosphate, in effect a rearrangement of the carbonyl group from the C-1 to the C-2 position in the ring structure, is not possible because this would require an oxygen atom on the C-2 position. Thus, deoxyglucose-6-phosphate is trapped in the cell, as the reverse reaction to deoxyglucose does not take place because of the low concentration of glucose-6-phosphatase in tumor cells.

After administration, FDG distribution can be depicted with PET. Areas of increased activity represent sites of

Fig. 8. Structural formula of 2-deoxy-2-fluoro-D-glucose as compared to D-glucose.

increased glucose metabolism. However, uptake of this radiopharmaceutical is not specific for malignant tissue and it has been demonstrated that it also localizes in areas of infection and inflammation (64, 65). In addition, there are several other tissues that exhibit a high glucose metabolism, including the brain and the heart (66). Also, FDG uptake in muscles could be considerably increased. Hence, complete rest is prescribed for patients before the scintigraphic examination (67). The uptake of FDG can be further reduced by fasting, thus causing hypoinsulinemia which is responsible for the decrease in glucose transporters of the muscle cell. Tissues with a relatively low FDG uptake are liver and gut, showing high glucose-6-phosphatase levels (66).

Uptake of FDG in tumor cells is influenced by a number of factors, of which we presumably only know a few. Besides biological factors, such as increased expression of glucose transporters and the presence of a highly active hexokinase isoform (described above), other influences, for example, are hypoxia and hyperglycemia.

FDG accumulation and hyperglycemia

FDG uptake into human cancer cells is inhibited by high media glucose levels, because of the competition between FDG and D-glucose for uptake into the malignant cell (68, 69). Simmons *et al.* (70) showed that GLUT-1 protein and mRNA levels were increased when fetal lung and muscle cells were cultured in low glucose media, while high glucose concentrations were associated with increased GLUT-1 protein and mRNA levels. This could be an adaptive response of the cell: a reduction in GLUT-1 transporters could serve as a method to keep glucose levels at a normal level in an hyperglycemic environment. On the other hand, an increase in GLUT-1 levels can give the tumor cell the possibility to survive in an hypoglycemic environment (71).

FDG accumulation and tumor metabolism in patients

Despite the gaps in our knowledge concerning FDG, FDG-PET is recognized to be an accurate, noninvasive tool for imaging a wide scale of different tumors. For a decade it has been suggested for the specific diagnosis and staging of cancer (72). More recently it is thought to be useful in the evaluation of cancer prognosis and efficacy of anticancer therapy (73), which is usually measured in terms of inhibition of proliferation, whereas FDG-PET measures the metabolic activity (74). For example, Cripa *et al.* (75) demonstrated that FDG uptake, represented by a standardized uptake value (SUV), was correlated with prognostic factors such as histopathological grading and p53 expression. Measuring metabolic activity is especially important since clinical studies on gliomas and lymphomas have shown that hypermetabolic tumors usually have a poorer prognosis than hypometabolic ones (66). Minn *et al.* (76) demonstrated that proliferative activity of tumors could be measured by FDG-PET. However, more recent research *in vitro* by Higashi *et al.* (77) showed that FDG accumulation represented the number of viable tumor cells, not their proliferative grade. In this regard, however, a number of aspects concerning the behavior and properties of the tumor cell should be addressed.

First, there is the fact that a tumor is not homogeneous, which means that malignant cells, premalignant cells and benign cells are all seen in the tumor, distributed in a scattered way, *e.g.*, clusters of malignant cells alternate with clusters of premalignant or even normal cells. Because scintigraphy cannot detect these clusters separately, the average energy demand of the cells within a tumor is depicted, which hampers the assessment of the real metabolic status of the tumor.

Second, tumors often induce an inflammatory response, resulting in the invasion of macrophages and formation of granulation tissue. Kubota *et al.* (78, 79) and Wijngaarden and Pauwels (64) mentioned that both macrophages and granulation tissue showed a higher uptake of FDG than viable tumor cells. In their articles, they summarized the components of a tumor: neoplastic tissue consisting of viable cells (high FDG uptake) and necrosis (no FDG uptake) and nonneoplastic tissue, divided into macrophages, young granulation tissue (high FDG uptake) and a scar (no FDG uptake). This phenomenon directly created the false-positive result in the work of Haberkorn (80). He studied patients with colorectal tumors: a higher FDG uptake after radiotherapy was seen at the tumor site, despite the success of the therapy.

Third, it is not clear how long after the administration of FDG one has to wait before the start of the scintigraphic examination. FDG uptake with time is most likely dependent on local parameters, such as mitotic activity and growth rate. Literature on this subject can hardly be found. Optimal posttherapeutic timing is also very important, thus reducing false-positive scans as a consequence of uptake of the above mentioned inflammatory cells.

Conclusions

Although FDG is the most widely used tumor imaging agent for positron emission tomography it is still worthwhile to continue investigations of this radiopharmaceutical in relation to tumor biology. In order to best use this

agent an understanding of its biochemical processing is needed in order to give it an appropriate clinical role. As described, upregulation of the membrane glucose transporters and enhanced production of glycolytic enzymes (hexokinase) are considered to be the main causes for the increased uptake and metabolism of glucose by tumor cells. Furthermore, the differences in behavior of glucose and FDG within tumor cells have been reviewed, as well as the factors that influence uptake of FDG and the limitations of FDG scintigraphy in relation to pathophysiology.

In this context it should be mentioned that at least three major questions in relation to its clinical use are still unanswered. First, which level of accumulation determines whether a cell is malignant or not. Second, what decrease in tumor FDG uptake must be achieved to assume effective treatment? Third, does FDG uptake measure the degree of aggressiveness of a tumor? Nevertheless, while it is clear that much basic and clinical validation remains to be done, our present clinical experience tells us that FDG-PET will continue to play an important role in the management of cancer patients.

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