Visualization of biochemical alterations. Oncological applications.

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I. INTRODUCTION

Nuclear Medicine is able of mapping and quantifying local activity, related with specific characteristics linked to some biochemical pathways involved in the malignant transformation or tumour proliferation. Some of these pathways are related with specific metabolic reactions, proteic synthesis, apoptosis, angiogenesis and tumoral hypoxia. For these purposes, Nuclear Medicine uses radiotracers, which are molecules that allow obtaining functional information through images, also named as molecular images.

II. GLUCOSE METABOLISM

In metabolic terms, the malignant transformation is characterized by increase of activity of glucose transporters, particularly GLUT-1, biosynthesis of a new class of transporters, increase of uptake glucose through the membrane, increase of glucose metabolism and also increase of normal glycolytic enzymes like hexokinase, phosphofructokinase and pyruvate dehydrogenase.

These alterations are under the use of fluorodoxyglucose (FDG), a glucose analog that has similar first metabolic steps in the glycolytic patway. If FDG is labelled with fluorine-18 (18F-FDG), we can visualize, using appropriate equipment, its increased uptake correlates with a high grade of malignancy or biologic aggressiveness. FDG metabolism reflects energy consumption in the tissues, being an index of cellular activity.

The biochemical mechanism involved in the utilization of FDG, allows the possibility of differentiate a tumoral lesion from post-radiotherapy necrosis, once necrosis has low metabolism. On the other hand, alterations in FDG uptake during the treatment can be an indicator of therapy response.

III NUCLEOSIDE METABOLISM

The S phase of the cellular cycle, which is the phase of deoxyribonucleic acid (DNA) synthesis, is related with the cell growth. In this phase, there are incorporation of nucleosides like thymidine and cytosine (known as pyrimidines bases) and adenosine and guanosine (known as purines bases) into DNA. The higher a tumour growth, the quicker it will incorporate nucleosides. The nucleoside more used by noninvasive medical imaging is the thymidine labelled with carbon-11 (11C-Thymidine).

A. 11C-Thymidine

The rate of cellular DNA synthesis is manly dependent of the tissue proliferative state. For image purposes, the thymidine molecule can be labelled with 11C, giving two different molecules, the methyl-11C-thymidine and the 11C-thymidine. After i.v. injection, both molecules are quickly uptaked by a great variety of human tumours, like lymphoma, carcinoma, brain tumours, sarcomas, lung, renal, and head-and-neck carcinoma.

B. Thymidine derivatives

The difficulties of using 11C, stimulate the development of several nucleoside analogues able to be labelled with fluorine-18. Among the different analogues developed, the more promising is the 3′-deoxy-3′-18F-Fluorothymidine (FLT). This radiopharmaceutical showed, both in animal models and in patients that have a better behaviour of FLT then FDG, being also more useful than FDG when therapy control is need.

C. Other nucleosides

Another nucleoside was proposed. One of the selected nucleoside derivatives was deoxyuridine. This molecule was labelled with several radioisotopes, like 131I, 124I or 77Br. The three deoxyuridine labelled molecules (77Br-deoxyuridine, 124I-deoxyuridine and 124I-deoxyuridine) are uptaked by tumoral tissue. However, the *in vivo* dehalogenation induces a high background activity, with the consequent low signal-noise relationship.

IV. AMINO ACIDS METABOLISM

Amino acids are markers of protein synthesis. In general terms, tumours need amino acids for protein synthesis, as metabolic fuel or to produce secretory products, that are poorly uptaked by the cells involved into the inflammatory processes. Amino acids image could give, with less false positive, better functional information with the perspective of have a better staging.

Some of de amino acids used are 11C-methyl-methionine (11C-MET), 11C-thyrosine (11C-TYR),18F-Fluoro phenylalanine and an artificial amino acid, the L-3-iodo-α-methyl tyrosine labelled with 123I (123I-IMT). Related with tyrosine, as it is an amino acid precursor of the melanin, the tyrosine derivatives might have importance for imaging patients with malignant melanomas.

V. TUMORAL HYPOXIA

In oncological situations, tumoural hypoxia is one of the most important factors implicated in resistance to radiotherapy and conventional chemotherapy. The information about the tumour hypoxia status, in order to predict the radiotherapy results or the eventual use of radiosensitizers, is now an important advance. The nuclear medicine with the possibility of labelled some molecules involved in the hypoxia cellular pathways, allows the possibility of having an image able to give qualitative and quantitative information about tumoural hypoxic condition. It is known that hypoxic tissues can uptake bioreductive molecules, like misonidazole that contains an imidazole group. The first molecules developed, with the objective of being molecular image agents were fluoromisonidazole (FMISO) and the fluoroerythronitroimidazole, both with a fluorine atom that allows labelling with 18F. Non-nitroimidazole compounds, like 4,9 diaza-3,3,10,10-tetramethyldode can-2,11-dione dioxime (HL-91) able to be labelled with 99mTc or Cu(II)-diacetyl-bis(N-4-methylthiosemicarbazone) (ATSM) able to be labelled with several copper isotopes, were developed.

VI. TUMORAL ANGIOGENESIS

Angiogenesis is the proliferation of endothelial and smooth muscle cells to form new blood vessels, a crucial factor of the metastatic process, with impact in two different points. Fist of all the new vessels provide the main route through which tumour cells exit from the primary tumour site and spread to other parts of the body, but, simultaneously, they supply the tumour cells with oxygen.

Several studies using cyclic peptides labelled with 18F, 99mTc, and 111In, to assess angiogenic status through an image, are now under investigation. If the high uptake shown in animal models is confirmed, these agents could be useful as image tracers for diagnose but also able to give information about tumour therapy response to $\alpha v\beta 3$ antagonists. EC-endostatin labelled with 99mTc (99mTc-EC-endostatin) is under study because can gives qualitative and quantitative information about tumour response to anti-angiogenic therapy.

Another important factor that plays an important role in cell division, cancer progression, angiogenesis and metastasis is Epidermal Growth Factor Receptor (EGFR). As many tumours express EGFR on their surface, also functional image using monoclonal antibodies that targets EGFR like the chimeric monoclonal antibody C225 labelled with 99mTc (99mTc-EC-C225) may give non-invasive information about EGFR expression, as well as information about clinical responses to anti-EGFR therapy or even about patient selection for treatment with C225.

VII. APOPTOSIS

Apoptosis, also known as programmed cell death, is now recognized as the major form of cell death after radiation. It seems that a dose of 1 to 5 Gy induces maximally apoptosis. It also seems that radiation-induced apoptosis appears quickly after irradiation, about 1–2 h, with a maximum value at 3 – 6 h after irradiation. In normal membranes, phosphatidyl serine is generally limited to the inner leaflet of the plasma membrane lipid bilayer. In cells that are in apoptosis, it is selectively and quickly translocated to the external leaflet, being exposed on the surfaces. Annexin V is an endogenous human protein, which is able to bind, with very high affinity to exposed phosphatidylserine, being considered as an apoptosis marker.

So far, annexin V has been labelled with iodine and with technetium after been coupled with diamide dimercaptide (N2S2) (99mTc-EC-annexin V) or hydrazinonicotinamide (99mTc-HYNIC-annexin V). This allows using it as image marker of apoptosis to monitor cell death dynamics and eventually, therapies effectiveness.

VIII. MULTIPLE DRUG RESISTANCE

Multiple drug resistance (MDR), is the development of cross resistance to a several citotoxic drugs, not related neither structural neither functionally after tumour exposure to an individual citotoxic drug. Actually, chemotherapeutic protocols involve combination of a group of drugs that acts on different cellular targets. This means that the drugs must be different in order to operate in different cellular metabolic pathways. If a malignant tumour expresses MDR, indicates that goes to show failure to chemotherapy. There are several group of transmembranar transport proteins related with multidrug resistance, such as P-glycoprotein, multidrug resistance-related protein (MRP), lung resistance-related protein (LRP), breast cancer related protein (BCRP) all of them ATP-binding cassette multidrug transporters.

99mTc labelled MIBI (99mTc-MIBI) and tetrofosmin (99mTc-TF] are lipophilic monocationic radiotracers, widely used for myocardial perfusion imaging, but that they are also substrates for both Pgp and MRP, being both used to assess Pgp/MRP-mediated drug resistance. Its tumoral accumulation is inversely proportional to Pgp expression. If chemosensitizers are used, both tracers are also able to evaluate the reversion of chemoresistance, once they increased its uptake, in comparison with the uptake before its use.

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