Journal of Labelled Compounds and Radiopharmaceuticals
J Label Compd Radiopharm 2007; **50**: 366–369.
Published online in Wiley InterScience
(www.interscience.wiley.com). DOI: 10.1002/jlcr.1269

JLCR

Short Research Article

Imaging the proliferative status of tumors with PET†

ROBERT H. MACH[1,*] and KENNETH T. WHEELER[2]

[1]Division of Radiological Sciences, Washington University School of Medicine, St. Louis, MO, USA
[2]Department of Radiology, Wake Forest University Health Sciences, Winston-Salem, NC, USA

Received 28 August 2006; Revised 4 January 2007; Accepted 20 January 2007

Abstract: The development of radiotracers for imaging solid tumors has recently focused on measuring a tumor's proliferative status. Two different strategies have emerged: (1) radiolabeled DNA precursors that measure DNA synthesis; and (2) radiotracers that label the sigma-2 receptor, a putative biomarker of proliferation. This paper provides a brief description of these two different methods of imaging tumor proliferation that are currently being developed in the field of positron emission tomography (PET). Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: PET; proliferative status; sigma-2 receptors

Introduction

Positron emission tomography (PET) is an *in vivo* imaging technique that is capable of measuring disease-associated changes at the molecular level. The use of PET in the field of oncology has largely centered on studies using the metabolic radiotracer, [^{18}F]FDG, which measures differences in glucose utilization between tumors and the surrounding normal tissue. Although this strategy is very useful in the diagnosis and staging of tumors, [^{18}F]FDG does not provide information that can be used to identify an appropriate strategy for treating the disease.

A recent strategy for imaging tumors involves the development of radiotracers that measure cellular proliferation and/or the proliferative status of solid tumors. The are a number of reasons why this information is useful in the treatment of cancer patients: (1) rapidly proliferating tumors are generally aggressive and have a high malignant potential that requires aggressive initial treatment; (2) rapidly proliferating tumors typically respond better to cell cycle specific agents (e.g. Ara-C and 5-fluorouracil) and hyperfractionated radiotherapy, while slowly proliferating tumors respond better to cell cycle nonspecific agents (e.g. cyclophosphamide and BCNU) and conventionally fractionated radiotherapy; and (3) a reduction in the proliferative status of a tumor can be used as an early predictor of the tumor's response to therapy.

There are two different strategies for imaging the proliferative status of solid tumors with PET. The first strategy involves the use of radiolabeled nucleoside analogs which utilize the salvage pathway of DNA synthesis for their uptake.[1] The second strategy involves the use of radiotracers that image the sigma-2 receptor status of solid tumors.

Discussion

Radiolabeled nucleoside analogs

Abbrogation of the ability to exit the cell cycle and form a natural quiescent state (G_0) is a key step in the transformation of a normal cell into a tumor cell (Figure 1). Therefore, one strategy that has been used to image tumor proliferation is to develop radiotracers that can image DNA synthesis, which occurs in the S-phase of the cell cycle. Since thymidine is the only nucleoside that is incorporated into DNA and not RNA, radiolabeled nucleoside analogs developed for PET imaging studies of tumor proliferation are based on either thymidine itself, or uracil analogs substituted in the 5-position of the pyrimidine ring so that they are incorporated into DNA, but not RNA (Figure 2). The

*Correspondence to: Robert H. Mach, Washington University, Radiology, 510 S. Kingshighway Blvd., St. Louis, MO 63110, USA.
E-mail: rhmach@mir.wustl.edu
Contract/grant sponsor: National Institutes of Health; contract/grant number: CA102869
†Proceedings of the Ninth International Symposium on the Synthesis and Applications of Isotopically Labelled Compounds, Edinburgh, 16–20 July 2006.

de novo pathway of DNA synthesis involves the conversion of uracil to thymidine via the enzymatic action of thymidylate synthase. Consequently, radiolabeled thymidine and its structural congeners must be incorporated into DNA via the salvage pathway of DNA synthesis. The first step in the salvage pathway of DNA synthesis involves the phosphorylation of the 5-hydroxy group of the furanose ring by the enzyme thymidine kinase-1 (TK1). The presence of a 3′-hydroxy group in FMAU, FBAU, and FIAU means that, like thymidine itself, these radiofluorinated analogs are incorporated into DNA and have the potential to measure DNA synthesis rates. On the other hand, [^{18}F]FLT, which lacks the 3′-hydroxy group required for elongation of the DNA polymer, is trapped in tumor cells as the corresponding 5′-phosphate analog. Thus, [^{18}F]FLT is not incorporated into DNA and can only indirectly estimate DNA synthesis rates.[1]

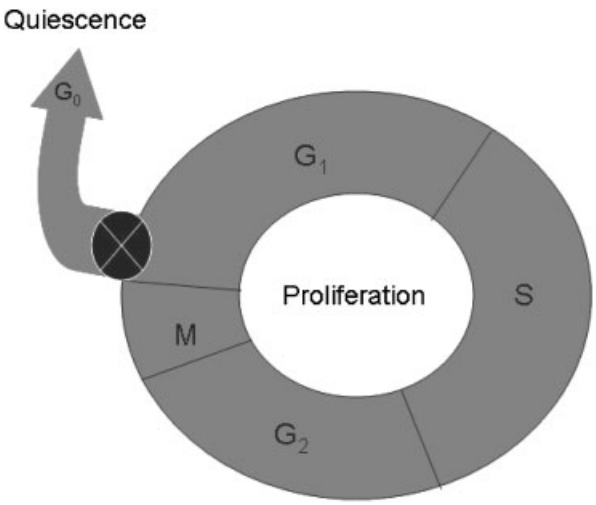

Figure 1 Stages of the cell cycle. Tumor cells often lose their ability to exit the cell cycle and enter a natural quiescent state after completing mitosis (M phase).

The initial imaging studies of tumor proliferation used [^{11}C]thymidine, which was labeled in either the 5-methyl group or in the carbonyl position designated by the asterisk in Figure 2. The difficult radiosynthesis, rapid *in vivo* metabolism, and complicated kinetic model needed to quantify cell proliferation has limited the utility of [^{11}C]thymidine as a PET radiotracer. Although the radiosynthesis of [^{18}F]FLT, [^{18}F]FMAU, [^{18}F]FBAU, and [^{18}F]FIAU also require multistep syntheses, the longer half-life of fluorine-18 (t= 110 min) *versus* that of carbon-11 (t= 20.4 min) indicates that these ^{18}F-labeled thymidine analogs have a higher likelihood of being used in routine PET imaging studies. Of the four ^{18}F-labeled thymidine analogs, [^{18}F]FLT has shown the greatest promise in tumor imaging studies.

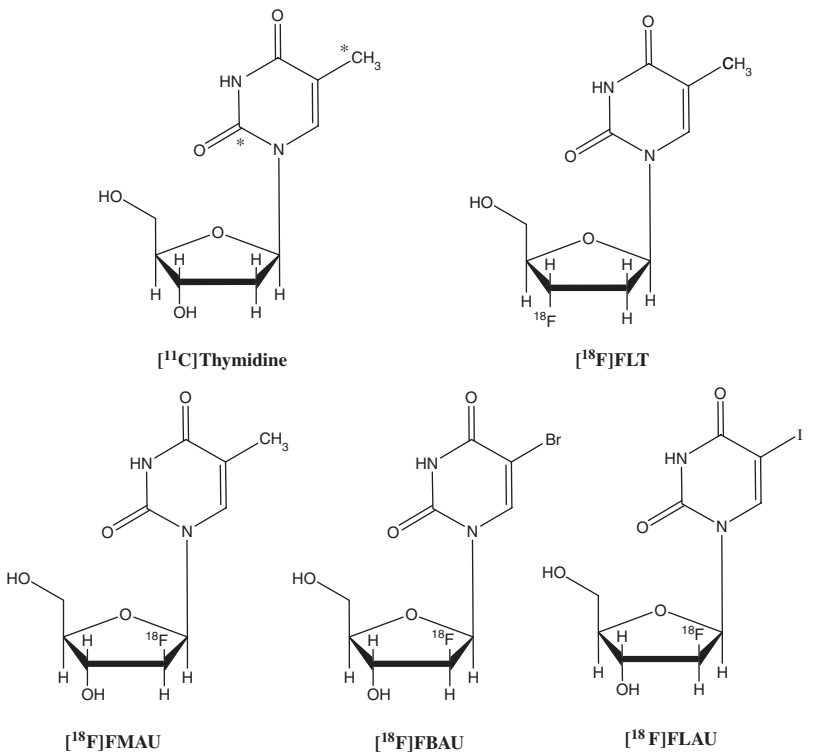

Figure 2 Radiolabeled DNA precursors for imaging proliferation.

DOI: 10.1002.jlcr

DNA synthesis via both the *de novo* and salvage pathways occurs during the S-phase of the cell cycle. Therefore, PET imaging studies with radiolabeled DNA precursors provide a 'snapshot' of the S-phase fraction of a tumor. Since tumor cells display asynchronous growth, cells within the tumor will exist in all four phases of the cell cycle. Agents which pulse label the cells that are only in S-phase will underestimate a tumor's proliferative status because the cycling cells in the G_1-, G_2- and M-phase will not be labeled. Furthermore, human tumors typically have highly variable potential doubling times (i.e. cell cycle times) on the order of one to several days. Since the length of the S-phase of mammalian cells is fixed at 8–10 h, only a small percentage of tumors cells with long cell cycle times will be in the S-phase for labeling with a radiolabeled DNA precursor. Therefore, a limitation of radiolabeled DNA precursors is that they will underestimate the true number of proliferating cells in a solid tumor, i.e. its proliferative status. A second limitation that has been observed in PET imaging studies with [^{18}F]FLT is the high uptake of the radiotracer in bone marrow, which could interfere with imaging bone metastases. Finally, concerns have been raised regarding the ability of PET radiotracers that image the *de novo* pathway of DNA synthesis to measure the response to chemotherapy.[2] In spite of these limitations, a number of promising PET imaging studies have been reported with [^{18}F]FLT, many of which display a positive correlation with Ki-67 expression, the present day 'gold standard' for estimating a tumor's proliferative status.[1]

Radiolabeled sigma-2 receptor radiotracers

A number of studies have reported that the sigma-2 receptor is a potential biomarker of the proliferative status of solid tumors. For example, studies using a tissue culture model of mouse mammary adenocarcinoma 66 cells have shown that the density of sigma-2 receptors is ~10 times higher in cycling proliferating (P) cells than in the corresponding noncycling quiescent (Q) tumor cells.[3] A subsequent study using solid tumor xenografts derived from the same tumor cell line demonstrated a positive correlation between the sigma-2 receptor density and the P:Q ratio measured by flow cytometry.[4,5] The agreement between the solid tumor and tissue culture data suggests that the expression of sigma-2 receptors is a reliable biomarker of the proliferative status of solid tumors. Therefore, radiotracers that target sigma-2 receptors should provide a measure of the P:Q ratio (i.e. the proliferative status) of a solid tumor.[3–5] Although these studies were conducted using a mouse mammary adenocarcinoma, the observation that there is a high density of sigma-2 receptors in a wide panel of human tumor cell lines suggests that this imaging strategy could be successful in many types of human tumors.[6]

Two different classes of compounds having a high affinity and selectivity for sigma-2 receptors have served as lead compounds for PET radiotracer development: (1) the 9-azabicyclo[3.3.1] nonan-3β-yl carbamates represented by **1** in Figure 3[7]; and (2) the conformationally flexible benzamide analogs represented by **2**, **3**, and **4** in Figure 3.[7–9] Although **1** has

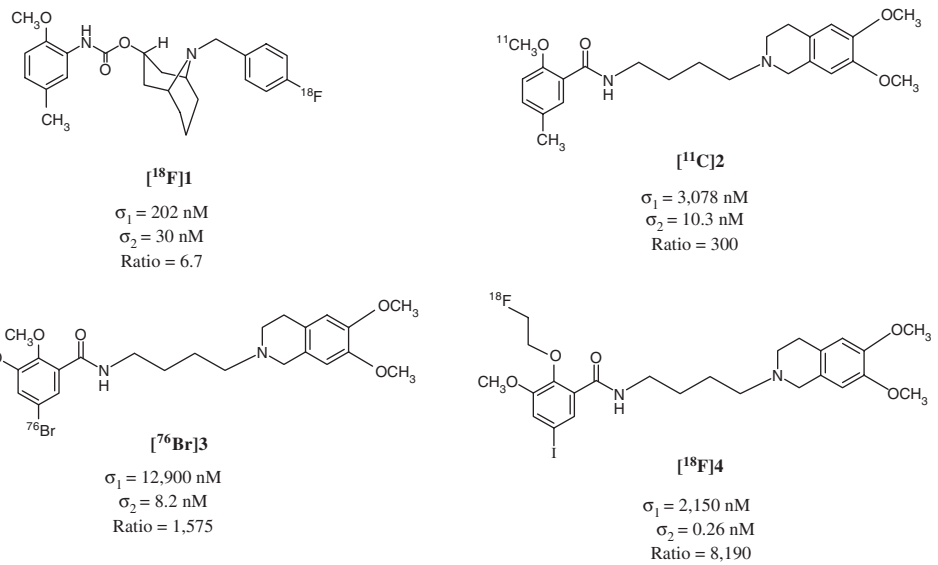

Figure 3 Structures of the conformationally flexible benzamide analogs used in PET imaging studies of the proliferative status of solid tumors.

DOI: 10.1002.jlcr

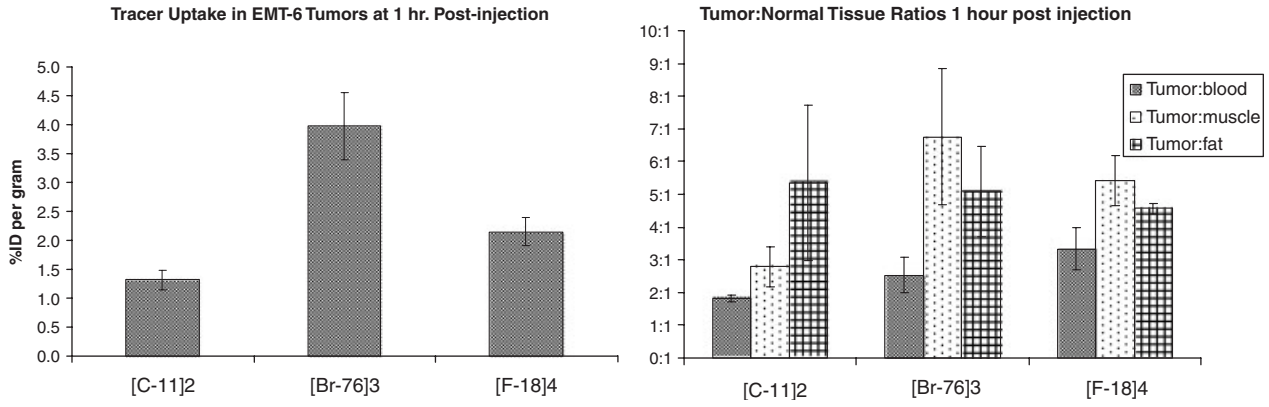

Figure 4 Tumor uptake (left) and tumor:normal tissue ratios of the radiolabeled conformationally flexible benzamide analogs in EMT-6 mouse breast tumors.

the advantage of being an ^{18}F-labeled radiotracer, the modest affinity for sigma-2 receptors, low sigma-2:sigma-1 selectivity ratio, and relatively low tumor:-normal tissue ratios indicates that it is not a good radiotracer for PET imaging studies of a tumor's proliferative status. However, Figure 4 demonstrates that this is not the case for the conformationally flexible benzamide analogs **2**, **3**, and **4**, because high tumor uptake and good tumor:normal tissue ratios are found for [^{11}C]**2**,[8] [^{76}Br]**3**,[9] and [^{18}F]**4**.[10]

The major limitation of the radiolabeled sigma-2 receptor analogs is the high uptake of the radiotracers in liver. This cannot be avoided since the liver contains a high density of sigma-2 receptors. Therefore, the utility of radiolabeled sigma-2 receptor ligands for measuring the proliferative status of solid tumors will be limited to tumors located above or below the abdominal cavity.

Conclusions

Two different imaging strategies have been developed for measuring tumor proliferation with PET: radiolabeled DNA precursors and radiolabeled sigma-2 receptor ligands. DNA precursors only label a small fraction of the cycling cells that are in S-phase at the time of the PET imaging study; thereby, frequently underestimating a tumor's proliferative status. However, sigma-2 receptor radiotracers heavily label all of the cycling cells in a tumor; thereby, providing a better estimate of its proliferative status. An accurate estimate of this parameter for either the whole or a region of the tumor is very important for selecting treatment strategies and/or assessing a tumor's response to treatment.

Acknowledgements

The sigma-2 receptor studies were funded by grants CA102869 awarded by the National Institutes of Health.

REFERENCES

1. Krohn KA, Mankoff DA, Eary JF. *J Clin Pharmacol* 2001; **41**: 96S–103S.
2. Been LB, Suurmeijer AJ, Cobben DC, Jager PL, Hoekstra HJ, Elsinga PH. *Eur J Nucl Med Imaging* 2004; **31**: 1659–1672.
3. Mach RH, Smith CR, Al-Nabulsi I, Whirrett BR, Childers SR, Wheeler KT. *Cancer Res* 1997; **57**: 156–161.
4. Al-Nabulsi I, Mach RH, Wang L-M, Wallen CA, Keng PC, Sten K, Childers SR, Wheeler KT. *Br J Cancer* 1999; **81**: 925–933.
5. Wheeler KT, Wang L-M, Wallen CA, Childers SR, Cline JM, Keng PC, Mach RH. *Br J Cancer* 2000; **86**: 1223–1232.
6. Vilner BJ, John CS, Bowen WD. *Cancer Res* 1995; **55**: 408–413.
7. Mach RH, Brown-Proctor C, Vangveravong S, Blair JB, Buchheimer N, Bottoms J, Wheeler KT. *Synth Applic Isot Label Comp* 2004; **4**: 157–160.
8. Tu Z, Dence CS, Ponde DE, Jones L, Wheeler KT, Welch MJ, Mach RH. *Nucl Med Biol* 2005; **32**: 423–430.
9. Rowland DJ, Tu Z, Xu J, Ponde D, Mach RH, Welch MJ. *J Nucl Med* 2006; **47**: 1041–1048.
10. Tu Z, Xu J, Li S, Jones LA, Wheeler KT, Welch MJ, Mach RH. *J Nucl Med* 2006; **47**(Suppl. 1): 90P.