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Imaging of Tumor Cell Proliferation using Radiofluorinated Ethyluracil and Deoxyadenosine

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= 국문초록 =

Radiofluorinated Ethyluracil과 Deoxyadenosine을 이용한 종양세포 증식의 영상화에 대한 연구

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목 적 : 종양세포의 증식을 평가하기 위해 radiofluorinated ethyluracil (FEU)과 deoxyadenosine analogue(FAD)를 합성하여 종양의 영상화를 시도하였다.

대상 및 방법 : 5-(2-Fluoroethyl)uracil ([^{18}F]FEU)은 2, 4-dimethoxy-5-(2-hydroxyethyl) pyrimidine을 K^{18}F와 처리한후 HBr로 가수분해하여 얻었으며 Fluorodeoxyadenosine은 adenosine의 triacetylated analogue를 K^{18}F와 처리하여 얻었다. 생물학적 조직분포는 유방암 세포(13762 NF, 100,000 cells per rat, im)를 쥐에 접종한후 0.5, 1, 2 및 4시간에 주요장기를 적출하여 %ID/g을 측정하고 자가방사영상은 방사성의약품 투여 45분후에 얻었다. PET 영상은 VX-2 종양을 접종한 가토를 이용하여 얻었다. In vitro cell proliferation assay는 사람의 말초단핵구를 이용하였다.

결 과 : In vitro assay상 [^{18}F]FEU는 세포증식시 DNA/RNA에 결합함을 시사하였다. [^{18}F]FAD와 [^{18}F]FEU의 종양/비종양 방사능 섭취비는 시간경과에 따라 증가하였으며 [^{18}F]FAD와 [^{18}F]FEU를 이용한 자가방사영상과 [^{18}F]FEU를 이용한 PET 영상에서 종양을 잘 관찰할 수 있었다.

결 론 : [^{18}F]FAD 및 [^{18}F]FEU를 이용하여 종양세포의 증식을 PET 영상에서 평가할 수 있으리라 사료된다.

Key Words : Fluoroethyluracil, Fluorodeoxyadenosine, Tumor Imaging, Autoradiography

INTRODUCTION

Noninvasive imaging assessment of tumor cell proliferation could be helpful in the evaluation of tumor growth potential and the degree of malignancy and could provide an early assessment of treatment response prior to changes in tumor size determined by computed tomography(CT), magnetic resonance imaging (MRI) or ultrasonography[1-3]. An understanding of tumor proliferative activity, in turn, could aid in the selection of optimal therapy by estimating patient prognosis and selecting the proper management.

Several efforts have been made to assess tumor proliferative activity. For instance, it has been reported that 2'-fluorodeoxyglucose ([^{18}F]FDG) uptake is an indicator of tumor proliferative activity[4-6]. Recently, Higashi et al. have shown that [^{18}F]FDG uptake is strongly related to the number of viable cells[7]. Another approach was to use radiolabeled thymidine as a tumor cell pro-

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liferative marker[8-11]. Thymidine, an analog of the pyrimidine nucleoside, is an essential building block of DNA, and is incorporated into DNA during the S-phase of cell proliferation. Synthesis and biological activity of many pyrimidine and purine nucleosides/nucleotides which can be incorporated into DNA/RNA has been reported [12-17]. Adenosine and 5-fluorouracil are also involved in DNA/RNA synthesis and are actively taken up by proliferating cells.

The synthesis of 9-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)adenine ([^{18}F]FAD), a threo or arabino configuration analog of 9-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)adenine (fluoroadenosine), and its biological activity have recently been reported, and the findings suggest that [^{18}F]FAD may be a good tumor imaging agent in the evaluation of tumor cell proliferation[18, 19].

While the synthesis and biological activity of many uracil analogs has been reported, to our knowledge, the radiosynthesis of [^{18}F]fluoroethyluracil and [^{18}F]fluorodeoxyadenosine and their ability to incorporate into DNA/RNA have not been investigated.

The purpose of this study was to develop the radiofluorinated ethyluracil(FEU) and the radiofluorinated deoxyadenosine analog(FAD) using a nucleophilic displacement reaction for noninvasive assessment of tumor proliferative potential by positron emission tomography(PET).

The significance of this proposal is to improve the understanding of the biological behavior of malignant tumors, which in turn will lead to a better prognostic evaluation, treatment selection and patient management.

MATERIALS AND METHODS

1. Synthesis of [^{18}F]Fluoroethyluracil [FEU] [2-Fluoroethyluracil]

In 2mL of dry pyridine, 184mg (1.0mmol) of 2,4-dimethoxy-5-(2-hydroxyethyl)pyrimidine[20] was dissolved with 210mg (1.1mmole) of p-toluenesulfonyl chloride. After stirring for 1 hour, the solvent was evaporated under reduced pressure. The resulting tosyl compound was used with K^{18}F and kryptofix in acetonitrile[21] to prepare the ^{18}F labeled compound which was then treated with HBr in acetic acid to remove the methyl protecting groups. The synthetic scheme is shown

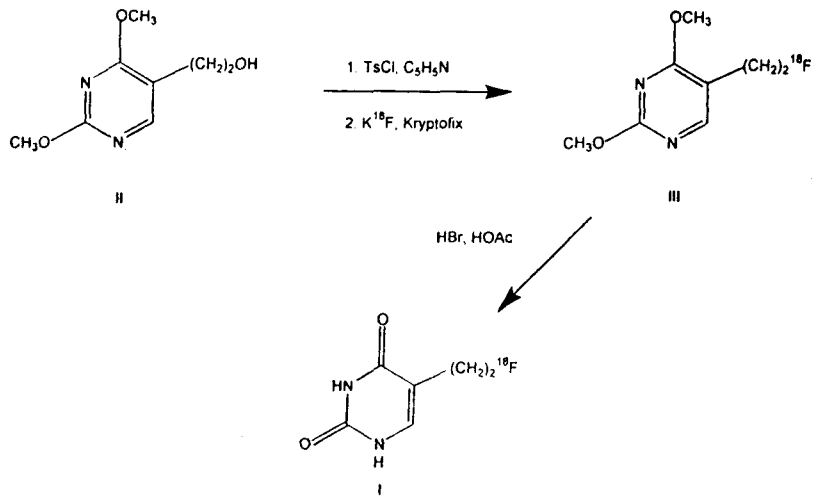

Fig. 1. Radiosynthesis of [^{18}F]Fluoroethyluracil.

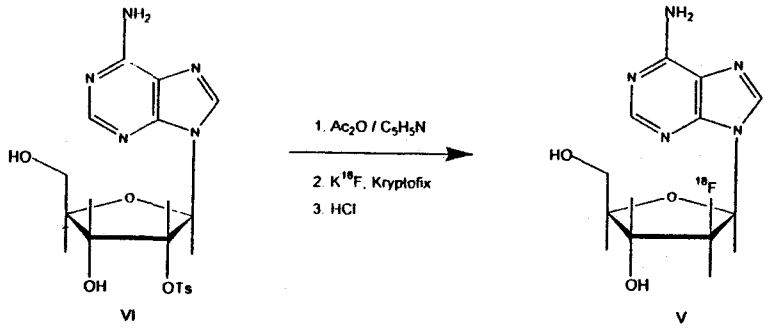

Fig. 2. Radiosynthesis of [^{18}F]Fluorodeoxyadenosine.

in Fig. 1.

2. Synthesis of [^{18}F]Fluorodeoxyadenosine [FAD][9-(2′-Deoxy-2′-fluoro-β-D-arabinofuranosyl)adenine]

To 100mg (0.238mmole) of 2′-O-p-toluenesulfonyladenosine dissolved in 5mL of THF, 2mL of acetic anhydride along with 2mL of pyridine was added and stirred overnight. Evaporation of acetic anhydride and solvents gave a material which was treated with K^{18}F and kryptofix in 3mL of acetonitrile. Hydrolysis with 2N HCl produced the desired material. The synthetic scheme is shown in Fig. 2.

3. In Vivo Biodistribution Study

Female F-344 rats were inoculated with breast tumor cells(13762 NF, 100,000 cells per rat, im., right leg). When the tumor reached 2cm in diameter, breast tumor-bearing rats were administered with [^{18}F]FEU or [^{18}F]FAD (5μCi/rat, iv., 3 rats/time interval), respectively. Tissue distribution was conducted at 30 min., 1, 2 and 4 hours. Percent of injected dose per gram of tissue weight (%IDG) was then determined.

4. Autoradiography of [^{18}F]FEU and [^{18}F]FAD

Female F-344 rats (n=3) were inoculated with breast tumor cells(13762 NF, 100,000 cells per rat, im., right leg). When the tumor reached 2 cm in diameter, each rat was given [^{18}F]FEU or [^{18}F]FAD (1.5 mCi, iv.), respectively. The rats were sacrificed at 45 min. postinjection. Each rat body was fixed a block of 4% carboxymethylcellulose. This block was then mounted on a Cryostat (LKB 2250 Cryo-microtome, Cambridge Instruments Company, Ijamsville, MD), and 50-60 μm coronal sections were made. These sections were then freeze-dried and placed over x-ray film(X-OMAT AR, Eastman Kodak Co., Rochester, NY) for 1 hour exposure.

5. PET Imaging Study

PET imaging studies were conducted using a VX-2 tumor-bearing rabbit. The rabbit was administered with [^{18}F]FEU (4mCi, iv.). Six consecutive 20 min. scans were acquired. The transaxial resolution of the scanner is 1.2cm(Posicam 6.5, Positron Co., Houston, TX).

6. In Vitro Cell Proliferation Assay

Human peripheral blood mononucleus cells (PBMC, 10^5 cells/well) were incubated with unlabeled FEU (0.01-1mM/well) for 3 days. On day 4, [^{3}H]thymidine was added (1μCi/well). To ascertain if FEU incorporates into DNA/RNA and is not due to cytotoxic effects, phytohemagglutinin (PHA, 0.1mg/well), a T-cell specific proliferating factor, was added with or without FEU, followed by the addition of [^{3}H]thymidine. The assay was

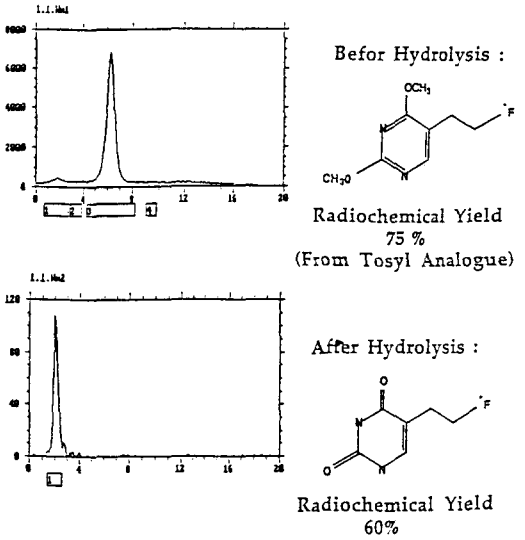

Fig. 3. Radio-TLC Analysis of [^{18}F]Fluoroethyluracil.

terminated 6 hours after adding [^{3}H] thymidine.

RESULTS

1. Radiosynthesis

[^{18}F]FEU was prepared from the tosyl precursor with a 30-40% yield. Radio-TLC analysis of the product before and after hydrolysis of [^{18}F]FEU with HBr using $CHCl_3$/MeOH (7:3) as mobile phase is shown in Fig. 3. Preparation of [^{18}F]FAD from the triacetyl analog of adenosine was similarly performed, followed by hydrolysis with 2N HCl, and had a 50-60% yield. The specific activity was estimated by HPLC in the range of 0.5-1Ci/μmol for both [^{18}F]FEU and [^{18}F]FAD (Fig. 1-3).

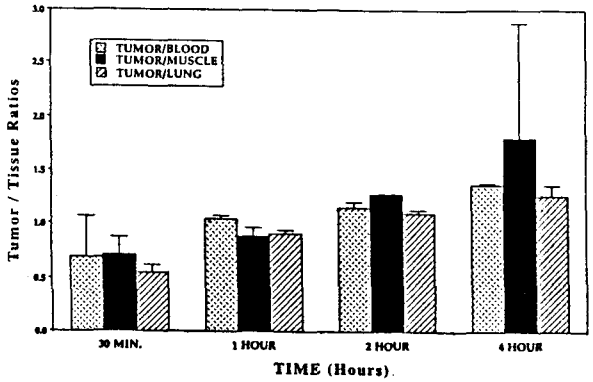

Fig. 4. Tumor-to-Tissue Ratios of [^{18}F]Fluoroethyluracil.

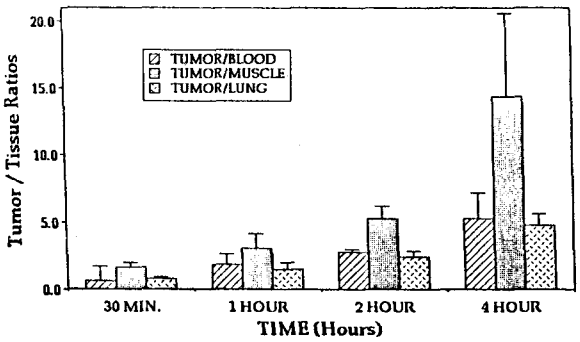

Fig. 5. Tumor-to-Tissue Ratios of [^{18}F]Fluorodeoxyadenosine.

Table 1. Biodistribution of [^{18}F]Fluoroethyluracil in Breast Tumor-Bearing Rats

Time	30 Min	1 Hr	2 Hr	4 Hr
Blood	0.422±0.0000	0.285±0.1579	0.233±0.0282	0.094±0.0278
Lung	0.531±0.1866	0.327±0.0176	0.244±0.0352	0.102±0.0210
Liver	0.749±0.0608	0.579±0.0588	0.525±0.0875	0.211±0.0454
Spleen	0.750±0.0966	0.590±0.0485	0.483±0.0636	0.219±0.0373
Kidney	0.359±0.0331	0.297±0.0116	0.228±0.0432	0.092±0.0203
Uterus	0.639±0.0975	0.408±0.0683	0.302±0.1634	0.221±0.0613
Bone	0.315±0.0406	0.479±0.1224	0.809±0.0549	1.124±0.1824
Tumor	0.294±0.1525	0.297±0.0283	0.268±0.0468	0.128±0.0397
Muscle	0.408±0.1025	0.335±0.0677	0.210±0.0351	0.071±0.1587

* Each data represents mean±standard deviation of percentage of injected dose per gram of tissue weight, N=3/time interval.

Table 2. Biodistribution of [^{18}F]Fluoroethyluracil in Breast Tumor-Bearing Rats

Time	30 Min	1 Hr	2 Hr	4 Hr
Blood	1.550±0.1120	0.519±0.1304	0.274±0.0358	0.132±0.0266
Lung	1.340±0.0464	0.619±0.0473	0.316±0.0167	0.142±0.0485
Liver	8.640±0.7330	4.960±0.3501	3.640±0.2086	2.090±0.2648
Spleen	1.060±0.0498	0.481±0.0466	0.338±0.0536	0.185±0.0270
Kidney	4.420±0.8438	1.730±0.0828	0.771±0.0836	0.366±0.0536
Muscle	0.613±0.0313	0.293±0.0379	0.146±0.0329	0.043±0.0106
Bone	9.750±0.3107	7.660±0.2015	14.640±0.1146	15.170±0.1542
Tumor	1.020±0.2539	0.886±0.2783	0.759±0.1225	0.661±0.1666

* Each data represents mean±standard deviation of percentage of injected dose per gram of tissue weight, N=3/time interval.

Table 3. In Vitro Cell Proliferation Assay in Peripheral Blood Mononuclear Cells(PBMC)1

Compound/Concentration	0.01 mM	0.1 mM	0.5 mM	1.0 mM
FEU	2,635.0±480.8	1,715.5±154.9	1,105.5±306.2	1,132.0±766.5
PHA+FEU	198,352±56,265	190,708±1,714.0	1,1763±405.9	1,275.5±314.7

1. Human peripheral blood mononuclear cells (PBMC, 10^5 cells/well) were incubated with unlabeled FEU (0.01-1 mM/well) for 3 days. On day 4, [3H] thymidine was added (1 μCi/well). To ascertain that FEU incorporates to DNA/RNA, not due to cytotoxic effects, phytohemagglutinin (PHA, 0.1mg/well) which is T-cell specific proliferating factor, was added with or without FEU, followed by adding [3H] thymidine. The assay was terminated 6 hours after adding [3H] thymidine. PHA (Positive Control) was 221,411± 59,094 and unstimulated (Negative Control) was 2,512.3±92.12

2. Biodistribution, Autoradiography and PET Imaging Studies

Tumor-to-blood and tumor-to-muscle count density ratios of both [^{18}F]FEU and [^{18}F]FAD increased as a function of time (Fig. 4, 5). [^{18}F]FAD showed a better image than [^{18}F]FEU due to higher tumor-to-nontumor tissue count density ratios. Bone uptake of both analogs significantly increased at 4 hours, suggesting that defluorination may have occurred(Table 1, 2). Autoradiograms of both [^{18}F]FEU and [^{18}F]FAD, and PET images of [18F]FEU, showed that the tumor could be well visualized at 45 min. post-

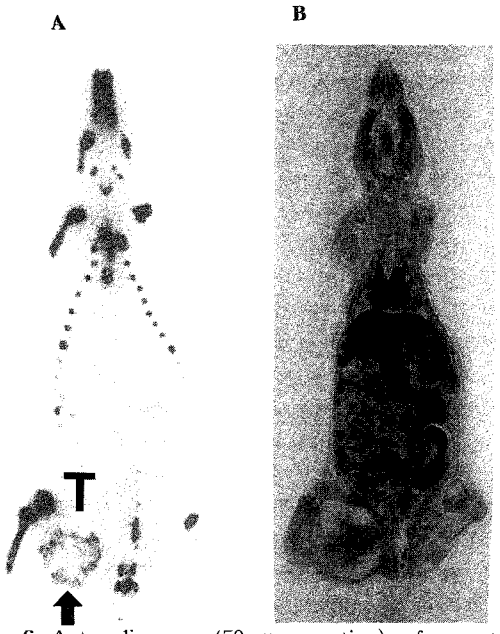

Fig. 6. Autoradiogram (50 μm section) of a rat administering [^{18}F]Fluoroethyluracil (1.5 mCi, iv) showed that the tumor could be visualized at 45 min. postinjection.

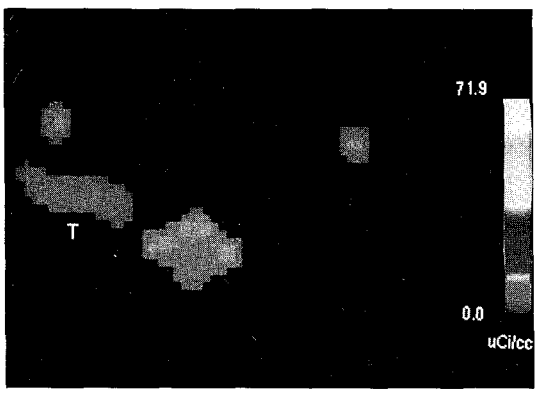

Fig. 7. PET image of a VX-2 tumor-bearing rabbit administering [^{18}F]Fluoroethyluracil (4 mCi, iv) showed that the tumor could be visualized at 45 min. postinjection. The tumor is located in right buttock area.

injection (Fig. 6-8).

3. In Vitro Cell Proliferation Assay

Cell proliferation assay indicates that [^{18}F]FEU competes with [^{3}H] thymidine for the binding sites in a dose-dependent manner(Table 3). This

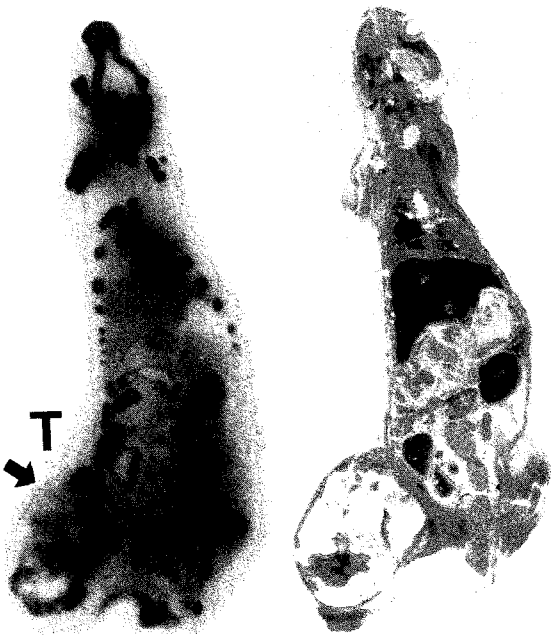

Fig. 8. Autoradiogram (50 μm section) of a rat administering [^{18}F]Fluorodeoxyadenosine (1.5 mCi, iv) showed that the tumor could be visualized at 45 min. postinjection.

data supports [^{18}F]FEU incorporation into DNA/RNA during cell proliferation.

DISCUSSION

In order to enhance biological activity and increase chemical or metabolic stability, fluorine substitution at the C2′ position of the sugar moiety (arabino configuration) has been widely investigated in durg research[15]. Replacement of the hydrogen by fluorine at this position alters biological activity and also increases chemical and metabolic stability[14].

[^{18}F]FAD is structurally closer to the 2′-deoxyadenosine analog(2′-deoxyarabinofuranosyladenine) than the corresponding unlabeled adenosine analog(arabinofuranosyladenine) due to the similarity in the Van der Waals radii between the C-H bond and C-F bond. Also, [^{18}F]FAD is expected to be more resistant to the purine

nucleoside phosphorylase than the corresponding unlabeled adenosine analog because 2′-fluoro-arabinosylpyrimidine has been shown to be stable to enxymatic cleavages[19]. To prevent undesired formation of intramolecular cyclization[14], the 3′, 5′-dihydroxy group of [^{18}F]FAD was protected by acetylation. By using readily accessible, commercially available 2′-tosyladenosine as a starting material the ^{18}F-labeled compound was obtained in 50-60% yield.

[^{18}F]FEU was prepared successfully by protecting the 2′ and 5′ positions with methyl groups. However, if these two positions were left unprotected, cyclization of the aliphatic chain to the pyrimidine ring during radiofluorination would have occurred[20].

Because it was been shown that even a small modification in the sugar moiety of the pyrimidine analog can dramatically change the biological activity[12], we performed a cell proliferation assay (in vitro) to test the incorporation of [^{18}F]FEU into DNA/RNA during cell proliferation. The data indicated that [^{18}F]FEU competes with thymidine for the binding sites in a dose dependent manner (Table 3).

Tumor to muscle ratios of [^{18}F]FAD at 2 and 4 hours postinjection were 5.2 and 14.3, respectively. Tumor to blood ratio at the same time intervals were 2.8 and 5.3, respectively. Tumor uptake(%IDG) was always greater than 0.66. These data were considered to be acceptable as a tumor imaging agent. Tumor to nontumor ratio of 1.7 on average was considered to be quite sufficient for tumor detection as determined by Willemsen A.T.M. et al. using [^{11}C]tyrosine and PET because the tumors are relatively close to the surface[22].

In contrast, tumor to nontumor tissue ratios of [^{18}F]FEU were much less than that of [^{18}F]FAD. The tumor to muscle ratio of [^{18}F]FEU was similar to the results reported by Atkins et al., where they have used [^{18}F] Fluorouracil(not fluoroethyluracil) as a tumor imaging agent[23]. The gradual increase in bone uptake was noted in both groups, more so in the [^{18}F]FAD group, and was thought to reflect that defluorination may have occurred. High accumulation of activity was also found in the liver and kidneys suggesting that liver appears to be the origin for metabolite production and that these metabolites of fluorinated nucleosides were excreted via the kidneys.

Tumors or portions of actively growing tumors were well visualized on autoradiography and PET images, indicating that [^{18}F]FAD and [^{18}F]FEU could be helpful in assessing tumor cell proliferation.

There are, however, many other radiopharmaceuticals used for assessment of tumor proliferation and/or metabolic activity[3].

Because, the radiopharmaceutical of choice will be determined not only by its biological behavior but also by its ease of preparation, as well as by the logistics of imaging, PET imaging must provide unique information that would not otherwise be obtainable by conventional morphologic imaging study, such as CT, MRI or ultrasonography. With this in mind, PET imaging studies using nucleoside/nucleotide analogs including [^{18}F]FAD or [^{18}F]FEU should be promising for the evaluation of tumor cell proliferation.

CONCLUSION

The high specific activity of ethyluracil and the adenosine analog could be achieved by using nucleophilic substitution reaction. The results suggest that both fluoroethyluracil ([^{18}F]FEU) and fluorodeoxyadenosine analog ([^{18}F]FAD) have sufficient potential for tumor imaging to evaluate tumor cell proliferation using PET. [^{18}F]FAD appears to be a better imaging agent than [^{18}F]FEU due to higher tumor-to-nontumor tissue

count density ratios.

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