Studies on $^{18}$F-labeled pyrimidines III. Biochemical investigation of $^{18}$F-labeled pyrimidines and comparison with $^3$H-deoxythymidine in tumor-bearing rats and mice

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Abstract. Metabolic studies of $^{18}$F-labeled 5-fluoro-2'-deoxyuridine (FdUrd), 5-fluorouridine (FUr) and 5-fluorouracil (FUra) were performed in tumor-bearing rats and mice. Also, the usefulness of $^{18}$F-FdUrd and $^3$H-deoxythymidine (dThd) for tumor detection was compared. In the tumor, 2 h after the injection of the $^{18}$F-pyrimidines, 3%–11% and 6%–14% of the $^{18}$F was present in the nuclear and microsomal fractions, respectively, and 17%–34% and 19%–24% of the $^{18}$F was incorporated into the acid-insoluble and nucleotide fractions, respectively. Of the three $^{18}$F-pyrimidines, $^{18}$F-FUrd demonstrated the highest incorporation rate, while $^{18}$F-FUra showed the lowest incorporation rate. The incorporation in the spleen, small intestine, and liver was less than that in the tumor. $^3$H-dThd and $^{18}$F-FdUrd were injected into the same mice. The $^3$H-dThd was accumulated in the spleen, small intestine, and tumor, and in these three tissues significant amounts of the $^3$H were incorporated into acid-insoluble materials. However, the clearance of $^{18}$F-FdUrd was slow in the tumor but rapid in the spleen and small intestine. In the autoradiograms of the tumor, $^{18}$F and $^3$H showed a slightly different distribution. Both distribution patterns were unchanged when the soluble materials were rinsed out with perchloric acid. For tumor detection, $^{18}$F-FdUrd gives the same information as radio-dThd, and further information can be obtained by positron-emission tomography.

Introduction

As positron-emitting radiopharmaceuticals allow a quantitative determination of the organ levels of drugs and their metabolites, as well as a noninvasive determination of the biochemical events, drugs which estimate the cell proliferation of tissues can assist in the choice of the correct diagnosis or therapy of tumors in humans.

5-Fluorouracil (FUra) is widely used as an antineoplastic agent in oncology, although its efficiency in patients is about 30% [7]. Two main mechanisms have been proposed for this agent. First, FUra is converted into ribonucleoside, phosphorylated, and then incorporated into RNA which causes a cytotoxic effect. Second, it is also converted into 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP) which irreversibly binds thymidylate synthetase. This reduces the thymidylate pool and inhibits DNA synthesis. Shani et al. have investigated whether $^{18}$F-labeled FUra can be utilized as a diagnostic agent for tumors [13, 14]. However, Crawford et al. [5] have shown that $^{18}$F-fluorouridine (FUrd) is more suitable than $^{18}$F-FUra for measuring the proliferation of tissues. On the other hand, $^{11}$C-labeled deoxythymidine (dThd), a natural precursor of DNA synthesis, has been synthesized enzymatically, and its feasibility has also been studied in tumor-bearing animals [3, 4]; however, this biosynthetic method of $^{11}$C-dThd is not generally employed for routine clinical study.

Our previous comparative study of the usefulness of $^{18}$F-FUra, $^{18}$F-FUrd, and $^{18}$F-5-fluoro-2'-deoxyuridine (FdUrd) for tumor detection confirmed that, of three pyrimidines, $^{18}$F-FdUrd is the most suitable tracer [1]. The degradation and excretion of these three $^{18}$F-pyrimidines has also been studied in rats [8]. In the present paper, biochemical investigations of the three $^{18}$F-pyrimidines were carried out in tumors and other organs of rats and mice. Also, the usefulness of $^{18}$F-FdUrd for tumor detection was compared with that of radio-dThd.

Materials and methods

Synthesis of $^{18}$F-FUra, $^{18}$F-FdUrd, and $^{18}$F-FUrd

The three $^{18}$F-labeled pyrimidines were synthesized using $^{18}$F-$^2$F as described previously [8].

Tumor-bearing animals

Transplantable-ascitic-hepatoma (AH109A)-bearing rats were prepared as has been previously described [1]. Transplantable mammary-carcinoma (FM3A or MM 48) cells were subcutaneously inoculated into C3H/He mice weighing 20–30 g. Two weeks after inoculation, tumors with a diameter of about 1 cm were used.

Subcellular fractionation

AH109A-bearing rats were injected with $^{18}$F-labeled pyrimidine (1–2 mCi) through a dorsal tail vein. At various times, rats were killed by cervical dislocation, and the tumor, liver, spleen, and small intestine were removed. All procedures were performed at 0°–4°C. Tissues (0.6–1.9 g) were homogenized in 4 ml 0.3 M sucrose, 1 m M ethylenediaminetetraacetic acid, and 10 mM Tris-HCl, pH 7.3 (buffer A), using a loose-fitting Teflon-glass homogenizer. The homogenate
was filtered through four layers of gauze and then washed with 2 ml buffer A. The filtrate was centrifuged at 3,000 rpm for 5 min, and the precipitate was washed twice by gentle dispersion in 2 ml buffer A followed by centrifugation. The precipitate was the nuclear fraction. The combined supernatant was centrifuged at 10,000 rpm for 10 min and then washed once in 2 ml buffer A. This precipitate was the crude mitochondrial fraction. The supernatant was further centrifuged at 35,000 rpm for 60 min and then washed once more. The final precipitate was the microsomal fraction, and the supernatant was the cytosol fraction. The cytosol fraction of the tumor was made up to 0.2 M in HClO₄ by the addition of 1 M HClO₄; it was then centrifuged at 3,000 rpm for 5 min. The pellet was suspended in 0.2 M HClO₄ and centrifuged. After one further wash, the pellet was an acid-insoluble material. The combined supernatant was an acid-soluble material. ¹⁸F-Radioactivity was measured with an autowell γ-counter (Packard Auto-Gamma 500C).

**Analysis of the metabolites**

Tissues (0.5–1 g) were homogenized in 2.5 ml of 0.2 M HClO₄. The homogenate was separated into acid-soluble and acid-insoluble materials as already described. The metabolites in the supernatant were analyzed according to the method of Chaudhuri et al. [2]. The supernatant was adjusted to pH 11 with 1 M KOH and centrifuged at 3,000 rpm for 5 min to remove the KClO₄. The supernatant was loaded on AG 1 × 8 (Bio-Rad HCOO⁻ form; 0.9 × 13 cm) and eluted stepwise with water (50 ml), 0.05 M HCOOH (50 ml), 1.5 M HCOOH (100 ml), 2.5 M HCOOH (50 ml), and 0.5 M HCl (50 ml). The base, nucleosides, dihydrofluorouracil, α-fluoro-β-alanine (FAla), and α-fluoro-β-guanidopropionic acid (FGPA) were eluted with 0.05 M HCOOH; α-fluoro-β-ureidopropionic acid (FUPA) was eluted with 1.5 M HCOOH; 5-fluorouridine monophosphate was eluted with 2.5 M HCOOH; nucleoside di- and tri phosphates were eluted with 0.5 M HCl.

**Biodistribution of ¹⁸F-FdUrd and ³H-dThd in tumor-bearing mice**

Tumor-bearing mice were injected with a mixture of ¹⁸F-FdUrd (about 100 µCi) and ³H-dThd (about 30 µCi; Amersham) through a dorsal tail vein. At various times, tissues were dissected and rinsed with saline. A part of the tissue solubilizer (NCS; Amersham), dissolved at 50 °C and mixed with 50°C C and breached with H₂O₂.

**Autoradiograms of tumor**

AH109A-bearing rat was injected with a mixture of ¹⁸F-FdUrd (10 mCi) and ³H-dThd (300 µCi). After 60 min, the tumor was dissected, frozen on dry ice, and cut into 20-µm-thick slices using an autocryotome. The slices were dried at 40°C on a hot-plate, and X-ray film (NMC, Kodak) was exposed to them for 6 h in order to image the ¹⁸F profile. After the ¹⁸F radioactivity had decayed, ³H film (Sakura) was exposed to the same slices for 21 days to image the ³H profile.

The neighboring slices were washed with 0.2 M HClO₄ to remove any soluble materials, and autoradiograms were also made for the ¹⁸F and ³H. The optical density on each autoradiogram was measured with a densitometer (Chromoscan 3; Joyce Loebl).

**Results**

**Subcellular distribution of the ¹⁸F-labeled pyrimidines**

Table 1 shows the subcellular distribution of the ¹⁸F-labeled pyrimidines in the tumor, spleen, small intestine, and liver. The study of the particle fractions of four tissues showed that the highest distribution of the ¹⁸F was in the tumor. With ¹⁸F-FdUrd, the proportion of the ¹⁸F in the tumor was larger in the nuclear fraction than in the microsomal fraction; with ¹³C-FUra, the relative proportions of the ¹⁸F in the two fractions were reversed. With ¹³C-FUra, 13.3% of the ¹⁸F was detected in the microsomal fraction, but only 2.9% of the ¹⁸F was detected in the nuclear fraction. About 80%–90% of the ¹³C in the particle fractions became acid insoluble. In the cytosol, 7%–13% of the total amount of ¹³C was present as an acid-insoluble material.

The spleen and small intestine showed a similar distribution pattern in the particle fractions to that of the tumor, but the distribution was lower. In the liver, 9.3% of the ¹³C was found in the microsomal fraction.

**Metabolites of ¹⁸F-labeled pyrimidines and ³H-thymidine in the organs**

The metabolites of the pyrimidines were analyzed in the tumor and other organs (Table 2). After the injection of ¹⁸F-FdUrd, the acid-insoluble ¹⁸F increased with time in the AH109A, and 25%–46% of the ¹⁸F was present in this fraction and the nucleotide fraction (2.5 M HCOOH and 0.5 M HCl fractions) over a period of 2 h. In the spleen, small intestine, and liver, the amounts of ¹⁸F present in these two fractions were 27.1%, 12.1%, and 4.6%, respectively. In the mouse tumor (MM48), 35%–50% of the ¹⁸F was also present in two fractions over a period of 2 h.

Two hours after the injection of ¹³C-FUra, ¹⁸F-FdUrd, and ¹³C-FUrd, 17%, 19%–24% and 34%, respectively, of the ¹⁸F was present in the acid-insoluble fraction of the AH109A, and about one-quarter of the ¹³C was found in the nucleotide fraction. However, even after 0.5 h, over 90% of the ³H-dThd in the MM48 was incorporated into the acid-insoluble and nucleotide fractions.

**Biodistribution and incorporation of ¹⁸F-FdUrd and ³H-dThd into acid-insoluble materials**

The biodistributions of the ¹⁸F-FdUrd and ³H-dThd in FM3A- or MM48-bearing mice are shown in Table 3 with the ratio of the acid-insoluble fraction. The distribution of the ¹⁸F-FdUrd was similar to previous results with rats [1]. The tumor uptakes in FM3A and MM48 were different.