III. 11 Metabolic Investigation of $^{18}$F-5-Fluorouracil, $^{18}$F-5-Fluoro-2'-Deoxyuridine and $^{18}$F-5-Fluorouridine in Rats

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Introduction

Fluorinated pyrimidines were effective antineoplastic agents in the treatment of certain epithelial tumors and their biological aspects in animals were investigated well.\(^1\) Recently investigations of $^{18}$F-labeled 5-fluorouracil ($^{18}$F-FUra) in tumor-bearing animals\(^2,3\) or human\(^4\) were reported. We also studied on tumor uptakes of $^{18}$F-FUra, $^{18}$F-5-fluoro-2'-deoxyuridine ($^{18}$F-FdUrd) and $^{18}$F-5-fluorouridine ($^{18}$F-Furd) in experimental animals.\(^5-7\) Although metabolic pathways of fluorinated pyrimidines had been confirmed using the $^{14}$C- or $^3$H-labeled one, no information with respect to $^{18}$F-labeled pyrimidines has been reported. In this report, we analyzed the metabolites of $^{18}$F-FUra, $^{18}$F-FdUrd and $^{18}$F-Furd in blood, bile and urine in rats.

Materials and Methods

$^{18}$F-FUra, $^{18}$FdUrd and $^{18}$F-Furd were synthesized as described previously.\(^8\)

All preparations were obtained with the high radiochemical purity (95-100 %). One of three pyrimidines was injected in Wister rats (200-250g) through a jugular vein. Blood was collected at various time intervals from a femoral artery and was centrifuged at 2000 rpm for 5 min to give serum. Bile or urine was collected continuously from bile duct or ureter. Three preparations were collected from individual rats. The fluorinated pyrimidines and their metabolites were analyzed on two high-performance liquid chromatographic systems: Radial-Pak C18 (8 mm x 10 cm)/10 mM (NH$_4$)$_2$HPO$_4$/4 ml/min and Aminex A-6 (4 mm x 30 cm)/0.2 N sodium citrate pH 4.25/1 ml/min.

Result and Discussion

Blood clearance and excretion in bile or urine:

Three pyrimidines showed similar blood clearance or excretion in bile or urine (Fig.1). The clearance in blood was very rapid for first 15 min and then slowed down. On the other hand the excretion in bile or urine reached maximum level at 10 or 20 min and lowered slowly. During first 2 hours 11-40 % and 0.7-3.5 % of $^{18}$F-radioactivity were excreted in urine and bile, respectively.

Analysis of metabolites in serum, bile and urine:

From the metabolic investigations of $^{14}$C- or $^3$H-labeled fluorinated pyrimidines, the degradation pathways of them are shown in Fig. 2.\(^1\) The metabolites of $^{18}$F-labeled each pyrimidines were analyzed on Aminex A-6 column and their HPLC profiles in serum were shown in Fig. 3. The peaks II, III, III', and V were
identified to be α-fluoro-β-analine(α-F-β-Ala), FdUrd, FUr and FUra, respectively, by comparison of their elution time with those of authentic samples. Because the proportion of the peak I was reduced by passing through an alumina column or precipitating as PbCl\(^{18}\)F in spite of the similar proportions of other peaks, the peak I contains fluoride anion and an unknown component(s), probably α-fluoro-β-ureidopropionate or α-fluoro-β-guanidopropionate (see Fig. 2). The fluoride anion could not detected in the metabolites on the studies using \(^{14}\)C- or \(^{3}\)H-labeled pyrimidines and should be added in the metabolic pathways. Although the peak IV was not identified, it is probably dihydrofluorouracil since it disappeared at 60 min. In vitro 18F-FdUrd showed no degradation in blood at 37°C for 2 hours. The proportion of each metabolites were shown in Fig 4, 5 and 6 with the elapse of time. In serum the pyrimidine nucleoside or base was rapidly degraded with a biological half-life of about 5 min and most of them disappeared at 60 min. In urine the metabolites reflected those in serum. However, in bile the main metabolites were detected in the peaks I and II(α-F-β-Ala), although a small amount of 18F-FUrd was detected only at 10 min. However, since in spite of the removal of all bile from the bile duct an analysis on metabolites of 18F-FdUrd in serum showed as similar results as shown in Fig. 3b and 5a, other tissues except for the liver might contribute somewhat degradation of fluorinated pyrimidines. In three preparations the proportion of α-F-β-Ala increased until 30 min and then decreased slowly and that of the peak I increased with time except for in bile at early time.

In this study, we confirmed that 18F-labeled pyrimidines degraded very rapidly mainly in the liver and excreted in bile and a considerable amount of pyrimidines and their metabolites was excreted in urine immediately after administration. These results represent the high uptakes of 18F-labeled pyrimidines in the liver and kidney. Further biological investigations will enable to estimate whether 18F-labeled pyrimidines are useful for the positron-emitting radiopharmaceuticals in nuclear medicine.

References

2) Shani J. and Wolf W., Cancer Res. 37, (1977) 2306.
Fig. 1. Blood clearance and excretion in bile or urine in individual rats after injection of $^{18}$F-FUra (a), $^{18}$F-PdUrd (b) and $^{18}$F-FUrd (c). Details were described in Materials and Methods. $^{18}$F-activity was expressed as the % of injected dose per g of blood (○), bile (□) or urine (△) at indicated times.

Fig. 2. Metabolic pathways of the fluorinated pyrimidines.
Fig. 3. Analysis of metabolites of $^{18}$F-labeled pyrimidines in serum on Aminex A-6 column. Serum at 10 min after injection of $^{18}$F-FUra(a), $^{18}$F-FdUrd(b) or $^{18}$F-FUrd(c) was analyzed.

Fig. 4. Metabolites of $^{18}$F-FUra in serum(a), bile(b) or urine(c). Each peak was expressed as the % of total recovered $^{18}$F-activity: ▲, I; △, II; □, IV; ●, V.
Fig. 5. Metabolites of $^{18}$F-FdUrd in serum(a), bile(b) or urine(c). Each peak was expressed as the % of total recovered $^{18}$F-activity: $\Delta$, I; $\triangle$, II; $\bigcirc$, III; $\square$, IV; $\bullet$, V.

Fig. 6. Metabolites of $^{18}$F-FUrd in serum(a), bile(b) or urine(c). Each peak was expressed as the % of total recovered $^{18}$F-activity: $\Delta$, I; $\triangle$, II; $\bigcirc$, III; $\square$, IV; $\bullet$, V.