STUDIES ON COMBINATION THERAPY WITH 1-(TETRAHYDRO-2-FURANYL)-5-FLUOROURACIL PLUS URACIL. II

Effects of Uracil on *In vitro* Metabolisms of 1-(Tetrahydro-2-furanyl)-5-fluorouracil and 5-Fluorouracil

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(Received May 23, 1980)

The effects of uracil on the *in vitro* metabolisms of 1-(tetrahydro-2-furanyl)-5-fluorouracil (FT) and 5-fluorouracil (5-UF) were studied.

^{3}H-FT and ^{3}H-uracil were both transferred smoothly from the mucosal to the serosal side of sacs of everted rat intestine, and the two absorptions did not interfere with each other.

After incubation of ^{3}H-FT with a rat liver homogenate, a small amount of 5-FU and about 9-fold more of its metabolites, 2-fluoro-3-ureidopropionic acid and 2-fluoro-β-alanine, were detected. The amount of 5-FU increased and the amounts of its metabolites decreased with increase in the concentration of added uracil, without any change in the extent of conversion of FT to 5-FU.

^{3}H-5-FU was taken up by AH 130 cells to a greater extent than ^{3}H-uracil. ^{3}H-5-FU was rapidly converted to 5-fluorouridine and 5-fluoronucleotides in the acid-soluble fraction, while most of the radioactivity in the acid-insoluble fraction was in RNA. The anabolic conversion of ^{3}H-5-FU in AH 130 cells were scarcely inhibited by 100-fold excess of uracil.

The inhibitory effect of 5-FU on the conversion of ^{14}C-formate into DNA-thymine and the resulting utilization of ^{3}H-thymidine were also not reversed by addition of uracil to the cell suspension.

INTRODUCTION

1-(2-Tetrahydrofuryl)-5-fluorouracil (FT)[1], a masked form of 5-fluorouracil (5-FU), is widely used as an oral antitumor agent.

Recently, FUJII *et al.*[2-4] found that coadministration of uracil enhanced the concentration of 5-FU in tumors and the resulting antitumor activity of FT, and suggested that for clinical trials the optimal molar ratio of uracil to FT should be 4. These findings were supported by the observations of TAGUCHI *et al.*[5-7] and KIMURA *et al.*[8] on the clinical pharmacology of UFT, a new antitumor agent, which is a mixture of FT and uracil in a molar ratio of 1:4.

Previously, we reported that the antitumor activity of UFT on various tumor systems was about 5 times that of FT alone and the same as that of 5-FU, and UFT was more effective than FT or 5-FU on LEWIS lung carcinoma and B 16 melanoma[9]. After oral adminstration of UFT, in which FT and uracil were bothrapidly absorbed from the intestine of AH 130 bearing rats, and no interaction between them was observed. After UFT administration to AH 130 bearing rats, the level of 5-FU in the blood increased transiently within 30 min and then decreased rapidly, and the 5-FU level in the tumor was maintained at a higher level and for a longer time than after FT adminstration. Higher levels of 5-FU, 5-fluorouridine (FUR) and 5-fluoronucleotides (F-nucleotides) were observed in the tumor than in normal tissues[10].

For antitumor activity FT must be converted to its active metabolite, 5-FU[11]. SAUNDERS *et al.*[12] observed that uracil reversed the growth inhibition of *Escherichia coli* B_s by FT and 5-FU. On the other hand, RICH *et al.*[13] reported that the growth inhibition of stock cultures of a human cervical carcinoma, *H. Ep.* #1 cells, by 5-FU could not be reversed with a 100-fold excess of uracil. REEVES *et al.*[14] also observed that the toxic effect of 5-FU on EHRLICH ascites tumor cell line, *ELD* cells, was not prevented by the presence of uracil, uridine or orotic acid. Similar findings on microorganisms and mammalian cells were reported by FUJII *et al.*[2]

This paper reports studies on the effects of uracil on the *in vitro* metabolisms of FT and its active metabolite, 5-FU.

MATERIALS AND METHODS

Chemicals : FT-6-^{3}H (^{3}H-FT, specific activity 27.8 mCi/mmole) was obtained from the Japan Radioisotope Association, Tokyo, Japan. 5-FU-6-^{3}H (^{3}H-5-FU, 2.0 Ci/mmole) and sodium formate-^{14}C (^{14}C-formate, 60.3 mCi/mmole) were purchased from the Radiochemical Centre, Amersham, England. Uracil-6-^{3}H (^{3}H-uracil, 23.3 Ci/mmole) and thymidine-6-^{3}H (^{3}H-thymidine, 23.0 Ci/mmole) were from the New England Nuclear Co., U.S.A. All other chemicals used in this study were of the highest grade available.

Intestinal transfer of FT and uracil : Male Donryu rats weighing 120~130 g, were starved for 18 hrs but allowed free access to water. Then they were killed by decapitation and the upper part of the small intestine was removed. Sace of everted intestine[15], 10 cm long, were filled with 1.5 ml of Krebs-Ringer phosphate buffer (pH 7.4). The sacs were suspended in 50 ml of the same fluid containing various concentrations of ^{3}H-FT with or without uracil, and ^{3}H-uracil with or without FT in 100-ml beakers. The beakers were incubated at 37° for 1 hr, bubbling 100% oxygen through the solution during the incubation. At intervals, aliquots (100 μl) of serosal solution were removed for measurement of radioactivity.

AH 130 cells : Rat ascites hepatoma, AH 130 cells, were maintained by intraperitoneal transfer in male Donryu strain rats. Cells were collected 7 day after inoculation, and used for experiments after washing them with phosphate-buffered saline (PBS) solution (pH 7.4).

Assay of FT metabolism *in vitro* : Male Donryu strain rats weighing 120~130 g were starved for 18 hrs and then decapitated. Their livers were rapidly perfused with cold saline and removed. The liver tissue was homogenized in 3 volumes of 50 mM potassium phosphate buffer (pH 7.4) containing 1.15% KCl, and the homogenate was centrifuged at 9,000×g for 15 min. The resulting supernatant was used as enzyme solution. All subsequent procedures were carried out at 4°. The incubation mixture in a final volume of 1.0 ml contained ^{3}H-FT (1.0 μCi), NADPH (2.5 μmole), Glucose-6-phosphate (G-6-P, 25 μmole), G-6-P dehydrogenase (15 units) and 0.8 ml of enzyme solution. Mixture with and without uracil were shaken at 37° for 1 hr. Then the reaction was stopped by adding cold methanol (1.0 ml), and the mixtures were centrifuged at 3,000 rpm for 15 min. Samples of 10 μl of the supernatant were applied to a thin layer chromatography plate (Kieselgel 60 F_{254} precoated, 5×20 cm, thickness 0.25 mm, Merck), and developed with chloroform : dioxane (1:1, v/v). Carrier FT, 5-FU, 2-fluoro-3-ureido propionic acid (F-UPA) and 2-fluoro-β-alanine (F-β-Ala) were applied to the TLC plate before the test sample. In this way, FT (Rf 0.75), 5-FU (Rf 0.25), F-UPA (Rf 0.01) and F-β-Ala (Rf 0.01) were separated.

Assays on intact cells : Freshly prepared AH 130 calls were suspended at 1×10^7 cells/ml in EAGLE's minimum essential medium (MEM) containing 10% calf serum and preincubated at 37° for 1 hr. Then labeled precursor with or without uracil was added, and the cells were incubated for various times. Then they were centriguged and washed with cold PBS solution until no radioactivity was detectable in the washing fluid. The acid-soluble and insoluble fractions of the cells were obtained by the method of SCHMIDT and THANNHAUSER[16]. The protein fraction was dissolved in NCS tissue solubilizer and other fractions were neutralized with 1 N KOH. The radioactivities of these fractions were measurred.

For assay of metabolites in the acid-soluble fraction, the neutralized samples were centrifuged, dried under a stream of nitrogen, and dissolved in 100 μl of 50% methanol. An aliquot (10 μl) was applied to a TLC plate of silica gel, and developed with a mixture of chloroform, acetone and water (25:50:2, v/v/v). In this way, 5-FU (Rf 0.54), 5-fluoro-2'-deoxyuridine (FUdR, Rf 0.43) and FUR (Rf 0.29) were separated from F-nucleotides, F-UPA and F-β-Ala, which remained at the origin. Uracil (Rf 0.36), 2'-deoxyuridine (UdR, Rf 0.26) and uridine (UR, Rf 0.14) were also located by the same procedures. (F-) pyrimidines were located by measuring the UV-absorption, while (F-) UPA and (F-) β-Ala were located by spraying the plates with EHRLICH reagent and ninhydrin, respectively. Same samples were applied to a TLC plate (Cellulose F, Merck, precoated, 5×20 cm, thickness 0.1 mm) and developed with a solvent composed of *n*-propanol, ammonia and water (6:3:1, v/v/v). (F-) Nucleotides with UV-

absorption moved together, were separated from other metabolites.

For measuring the incorporation of ^{14}C-formate into DNA in the cells, DNA was rehydrolysed by the method of HERSHEY *et al.*[17], and an aliquot of the hydrolyzate was applied to a silica gel TLC plate, and developed with ethyl acetate: ethanol: water (4:2:1. v/v/v). Thymine (Rf 0.62) was separated from adenine (Rf 0.33), cytosine (Rf 0.18) and guanine (Ru 0.06). DNA and DNA-thymine were assayed by the method of BURTON[18] and measuring the UV-absorption at 264.5 nm, respectively.

Measurement of radioactivity : Radioactive spots on the TLC plate were scraped off, placed in a vial, extracted with 0.5 ml of water for 2 hrs and then mixed with 10 ml of dioxane-system scintillator. Other compounds were incubated in 0.5 ml of NCS tissue solubilizer at 50° for 1 hr, neutralized with acetic acid, and mixed with 10 ml of toluene-system scintillator. The redioactivity of each sample was measured in an Aloka LSC 673 liquid scintillation spectrometer.

RESULTS

Effect of uracil on intestinal transfer of FT : Since FT and uracil are usually administered orally, their absorptions from rat intestine were examined by the everted sac method[15]. When the initial concentration of ^{3}H-FT on the mucosal side was 2.0 μCi (equivalent to 200 μg)/ml, its concentration in the serosal side increased linearly with the incubation time, being 11.8 μg/ml after 15 min, 28.6 μg/ml after 30 min and 79.2 μg/ml after 60 min (Fig. 1). ^{3}H-uracil was also transferred across the intestinal wall during incubation; when its initial concentrations on the mucosal side were 1 μCi (equivalent to 112 μg), 2 μCi (224 μg), 5 μCi (560 μg) and 10 μCi (1,120 μg)/ml, its concentrations on the serosal side after 60 min were 52.6, 98.0, 235.6 and 376.4 μg/ml, respectively (Fig. 2). The transfer of ^{3}H-FT (200 υg/ml) was not affected by the addition of uracil (112~1,120 μg/ml) and *vice versa*. These findings suggest that FT and uracil may be absorbed by different transport systems.

Effect of uracil on the metabolism of FT by a rat liver homogenate: The above results show that FT and uracil are easily and repidly absorbed from the intestine. Thus they are presumably transferred to the liver *via* the portal vein after the oral administration of FT combined with uracil. The

Fig. 1 Effect of uracil on the absorption of ^{3}H-FT be everted sacs of rat intestine
Mucosal side ; ^{3}H-FT : 2.0 μCi (1.0 μmole)/ml
Uracil : 1.0~10 μmoles/ml
Serosal side ; 1.5 ml of Krebs-Ringer phosphate buffer (pH 7.4)

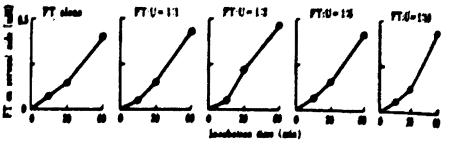
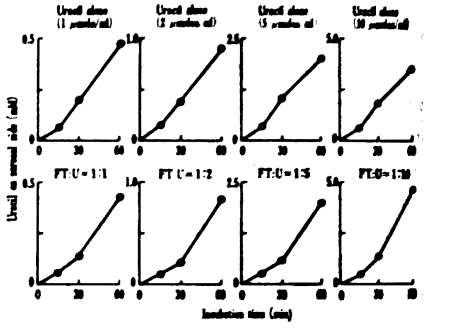


Fig. 2 Effect of FT on uptake of ^{3}H-uracil by everted sacs of rat intestine
Mucosal side ; ^{3}H-uracil : 1.0~10 μCi (1.0~10 μmoles)/ml
FT : 1.0 μmole/ml
Serosal side ; 1.5 ml of Krebs-Ringer phosphate buffer (pH 7.4)



conversion of FT to 5-FU[11] and the degradation of 5-FU to F-UPA and F-β-Ala[19,20] both occur mainly in the liver. So, the effect of uracil on the metabolism of FT by a rat liver homogenate was investigated. After incubation of ^{3}H-FT (1.0 μCi, 0.5 μmole/ml) with a rat liver homogenate in the presence of NADPH plus its generating system for 1 hr, 1.3 nmole/ml of 5-FU and 10.8 nmole/ml of its further metabolites (F-UPA and F-β-Ala) were detected. This means about 90% of the 5-FU from FT was degraded under these conditions. In the presence of uracil, the concentration of 5-FU increased and the concentrations of its catabolites decreased in proportion to the concentration of uracil, being 11.2 nmole/ml and 1.8 nmole/ml, respectively, with 5 μmole/ml of uracil. However, no change of the extent of conversion of FT to 5-FU was observed even at a 10 times higher concentration as FT. This observation suggests that uracil does not affect the conversion of FT to 5-FU, but inhibits the degradation of 5-FU to further metabolites. This effect should increase the antitumor activity of FT by increasing the

Table 1 Effect of uracil on the metabolism of ^{3}H-FT in a rat liver homogenate

nmoles/ml

	FT	5-FU	F-UPA F-β-Ala
^{3}H-FT	467.8	1.3	10.8
^{3}H-FT+uracil (1 : 1)	458.8	5.1	6.6
(1 : 2)	459.7	6.9	4.4
(1 : 3)	472.6	8.6	3.8
(1 : 4)	468.8	9.6	3.4
(1 : 5)	470.9	9.3	2.6
(1:10)	473.4	11.2	1.8

^{3}H-FT : 1 μCi (0.5 μmoles), uracil : 0.5~5 μmoles
NADPH : 2.5 μmoles, G-6-P : 25 μmoles, G-6-P dehydrogenase : 15 units
Supernatant (9,000 g, 15 min) of 25% rat liver homogenate : 0.8 ml
Incubation : 37°, 60 min, with shaking

concentration of 5-FU.

Incorporation of 5-FU into AH 130 cells in the presence of uracil: As reported previously, the levels of 5-FU and uracil in the blood of AH 130 bearing rats were maximized 30 min after oral administration of UFT at 48.6 mg/kg (FT: 15 mg/kg, uracil: 33.6 mg/kg) and these peak concentrations were 0.43 μg/ml and 13.1 μg/ml, respectively[10]. It seemed possible that uracil might interfere with the incorporation of 5-FU into tumor cells. So, we next examined the effect of uracil on the incorporation of 5-FU into AH 130 cells. AH 130 cells (1×10^7 cells/ml) were incubated with ^{3}H-5-FU (0.1 μCi/ml) or ^{3}H-uracil (0.1~10 μCi/ml) in EAGLE's MEM containing 10% calf serum. After incubation for various times, the cells were centrifuged and washed with cold PBS solution. The pellet of cells was then dissolved in 0.5 ml of NCS tissue solubilizer and its redioactivity was measured. The incorporations of ^{3}H-5-FU into the cells in the presence of uracil are shown in Fig. 3. During incubation, ^{3}H-5-FU was rapidly taken up and the total radioactivity in the cells after 60 min was about twice that after 15 min. The up take of ^{3}H-5-FU was not affected by the presence of uracil even at 100 times the concentration as ^{3}H-5-FU. The incorporation of ^{3}H-uracil was similar to that of ^{3}H-5-FU and was also not affected by 5-FU (Fig. 4).

Effect of uracil on the metabolism of 5-FU in AH 130 cells : Table 2 ahowed the distribution of radioactivity in the cells after incubation of a cell

Fig. 3 Effect of uracil on incorporation of ^{3}H-5-FU into AH 130 cells
AH 130 cells (1×10^7cells/ml) were incubacted in EAGLE's MEM containing 0.1 μCi (0.01 μmole)/ml of ^{3}H-5-FU with or without 0.01~1.0 μmole/ml of uracil.

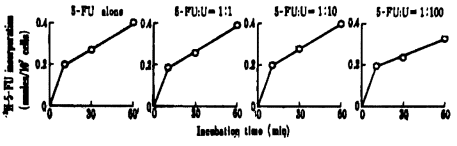
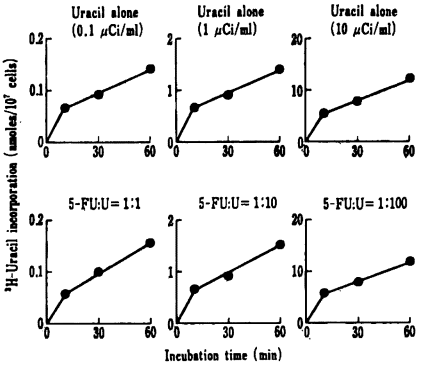


Fig. 4 Effect of 5-FU on incorporation of ^{3}H-uracil into AH 130 cells
AH 130 cells (1×10^7cells/ml) were incubated in EAGLE's MEM containing 0.1~10 μCi (0.01~1.0 μmole)/ml of ^{3}H-uracil with or without 0.01 μmole/ml of FT.



suspension (1×10^7 cells/ml) with ^{3}H-5-FU (5 μCi/ml) for 1 hr. About 70% of the radioactivity taken up into the cells was present in the acid-soluble fraction, and of this redioactivity 20% was present in 5-FU, 5% in FUR and 75% in F-nucleotides. Scarcely any activity was found in FUdR. The composition of the metabolites in the acid-soluble fraction suggested that the 5-FU incorporated into the cells was rapidly phosphorylated. On the other hand, about 90% of the radioactivity in the acid-insoluble fraction was present in RNA, not DNA. These results are consistent with the findings of HEIDELBERGER *et al.*[21~28] on EHRLICH ascites carcinoma. The incorporation of ^{3}H-FU into the cells and its anabolism were similar in the presence and absence of uracil. The presence of uracil at 100-fold the concentration of ^{3}H-5-FU increased the amount of 5-FU and decreased that of F-nucleotides in the acid-soluble fraction, and decreased the incorporation of radioactivity into RNA, slightly.

The distribution of radioactivity of ^{3}H-5-FU in

Table 2 Effect of uracil on the anabolism of ^{3}H-5-FU in intact AH 130 cells

	^{3}H-5-FU	^{3}H-5-FU+uracil (molar ratio)		
	alone	(1 : 1)	(1 : 10)	(1 : 100)
Inside cells	381.6	348.6	388.1	359.0
Acid-soluble fraction				
5-FU	58.5	54.2	64.7	65.2
FUR	13.7	17.6	17.4	24.5
FUdR	0	0	0.1	0.3
F-nucleotides	228.4	194.5	206.4	166.7
Acid-insoluble fraction				
Lipid	6.5	5.0	5.6	6.5
RNA	101.4	97.2	103.5	84.8
DNA	1.6	0.6	0.9	1.0
Protein	2.7	2.2	2.2	2.0

Values are dpm$\times 10^3/10^7$ cells.
Substrate : 5 μCi (0.5 Ci/mmole)/ml
AH 130 cells : 1×10^7 cells/ml of EAGLE's MEM containing 10% calf serum

Fig. 5 Distribution of radioactivity of ^{3}H-5-FU and ^{3}H-uracil in AH 130 cells
Each precursor (100 mCi/mmole) was incubated with AH 130 cell suspension (1×10^7 cells/ml of EAGLE's MEM containing 10% calf serum)

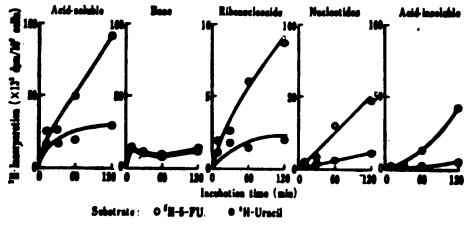
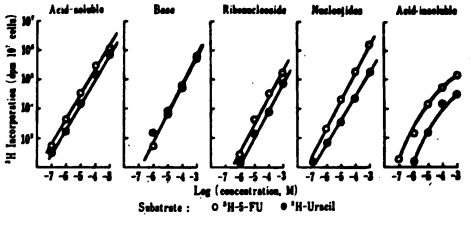


Fig. 6 Distribution of radioactivity of ^{3}H-5-FU and ^{3}H-uracil in AH 130 cells
Each precursor (100 μCi/mmole) was incubated with AH 130 cell suspension (1×10^7cells/ml of EAGLE's MEM containing 10% calf serum)



the cells was compared with that of ^{3}H-uracil. The total radioactivity of ^{3}H-5-FU in the acid-soluble fraction was about 3 times that of ^{3}H-uracil. In the base, the radioactivities of ^{3}H-5-FU and ^{3}H-uracil were the same, but in nucleotides, radioactivity of ^{3}H-5-FU was about 4~5 times higher than that of ^{3}H-uracil. Moreover, in the acid-insoluble fraction, the radioactivity of ^{3}H-5-FU was greater than that of ^{3}H-uracil. These findings suggest that uracil has little reciprocal effect on 5-FU.

Effect of uracil on inhibition of DNA synthesis by 5-FU : 5-FU inhibits the conversion of ^{14}C-formate to the methyl group of DNA-thymine in EHRLICH ascites tumor cells *in vitro*[29] and *in vivo*[30], Moreover, 5-FU stimulated the incorporation of ^{3}H-thymidine into DNA-thymine in all tissues tested[34]. For investigating the effect of uracil on the inhibition of DNA synthesis by 5-FU, we measured the incorporation of ^{14}C-formate into DNA-thymine and of ^{3}H-thymidine into AH 130 cells incubated with or without uracil. The conversion of ^{14}C-formate to DNA-thymine was 2,123 dpm/μg of thymine in the control group. This conversion was inhibited by 5-FU in proportion to the concentration of 5-FU; the radioactivity was 923 dpm/μg (*ca.* 50% of control) with 1×10^{-5}M and 222 dpm/μg (*ca.* 10% of control) with 1×10^{-4}M. In the presence of uracil at 1×10^{-5}~1×10^{-3}M, the inhibition of

Table 3 Effect of uracil on inhibition of ^{14}C-formate incorporation into DNA thymine in AH 130 cells by 5-FU
dpm/μg of DNA thymine

	10 min	30 min	60 min
Control	439 (100)*	1,250 (100)*	2,449 (100)*
5-FU (1×10^{-5}M)	341 (78)	535 (43)	1,165 (48)
5-FU+uracil (1 : 1)	193 (44)	600 (48)	887 (36)
(1 : 10)	284 (65)	593 (47)	747 (30)
(1 : 100)	308 (70)	885 (71)	1,090 (45)
uracil (1×10^{-3}M)	515 (117)	1,004 (84)	1,971 (80)

* per cent of control
^{14}C-formate : 1.0 μCi, 5-FU : 1×10^{-5}M, uracil : 1×10^{-5}~1×10^{-3}M
AH 130 cells : 1×10^7 cells/ml of EAGLE's MEM containing 10% calf serum

Fig. 7 Effects of 5-FU and uracil on incorporation of ^{14}C-formate into DNA thymine in AH 130 cells

AH 130 cells (1×10^7cells/ml) were preincubated with 5-FU or uracil for 60 min, and then incubated with ^{14}C-formate (60.3 mCi/mmole) at 1.0 μCi/mi for 30 min.

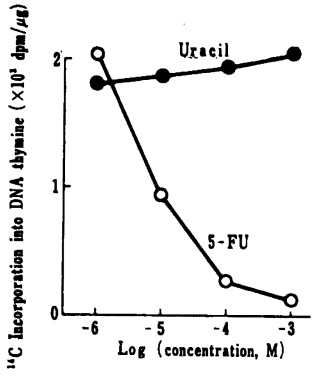
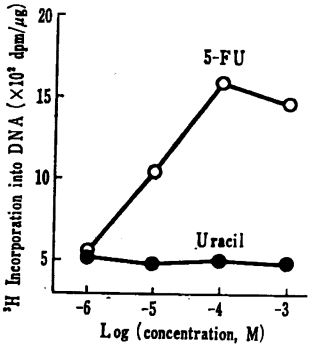


Fig. 8 Effects of 5-FU and uracil on incorporation of ^{3}H-thymidine into DNA in AH 130 cells

AH 130 cells (1×10^7cells/ml) were preincubated with 5-FU or uracil for 60 min, and then incubated with ^{3}H-thymidine (23.0 Ci/mmole) at 1.0 μCi/ml for 30 min.



incorporation of ^{14}C-formate into DNA-thymine by 1×10^{-5}M of 5-FU was scarcely affected (Table 3). Fig. 8 shows the incorporation of ^{3}H-thymidine into DNA in AH 130 cells at various concentrations of 5-FU and uracil. The radioactivity of the control was 480 dpm/μg of DNA, and those of the groups with 1×10^{-5}M and 1×10^{-4}M of 5-FU were 1,070 dpm/μg (*ca.* 2-fold of control) and 1,573 dpm/μg (*ca.* 3-fold of control), respectively. This accumulation of ^{3}H-thymidine in DNA in the presence of 5-FU was well correlated with inhibition of the incorporation of ^{14}C-formate into DNA-thymine shown in Fig. 7. Uracil had no significant effect

Table 4 Effect of uracil on enhancement of incorporation of ^{3}H-thymidine into DNA in AH 130 cells by 5-FU

dpm/μg of DNA

	10 min	30 min	60 min
Control	99 (100)*	541 (100)*	1,220 (100)*
5-FU (1×10^{-5}M)	118 (114)	732 (135)	1,546 (127)
5-FU+uracil (1 : 1)	128 (130)	659 (122)	1,721 (141)
(1 : 10)	117 (118)	706 (131)	1,576 (129)
(1 : 100)	124 (126)	737 (136)	1,572 (129)
uracil (1×10^{-3}M)	100 (101)	510 (94)	1,174 (96)

* per cent of control

^{3}H-thymidine : 1.0 μCi, 5-FU : 1×10^{-5}M, uracil : $1\times10^{-5}\sim1\times10^{-3}$M

AH 130 cells : 1×10^7 cells/ml of EAGLE's MEM containing 10% calf serum

in this experiment (Table 4). These results indicate that the inhibition of DNA synthesis by 5-FU is not reversed by uracil.

DISCUSSION

After oral administration of FT to rats, its concentration in the blod reaches a peak within 1 hr[25]. Uracil is absorbed by an active transport system[26]. But the intestinal absorption of FT, which occurs mainly in the jejunum, seems to be due to passive diffusion, because it is not inhibited by ouabain[27]. FT was transferred from the mucosal to the serosal side of sacs of rat intestine whether uracil was present or absent, and the linear dependence of the intracellular concentration on the extracellular concentration also suggests that the transfer occurs by simple diflusion (Figs. 1 and 2).

Previously, FUJII *et al.*[11] found that FT is canverted to 5-FU in the presence of NADPH, mainly by the microsomal fraction of rat liver, and they suggested that microsomal electron transport is concerned with this reaction. OHIRA *et al.*[28] observed the difference spectra of FT and rat microsomes, and reported that the spectral dissociation constant (Ks) is 26.3 mM. This Ks value is about 25 times more than that of cyclophosphamide (Ks =1.05 Mm), which is also activated by the microsomal drug-metabolizing enzymes. The liver is probably the main site of catabolism of 5-FU[19,20]. 5-FU is metabolized more extensively when it is

administered orally than when it is injected intravenously, and about 85~95% of the 5-FU reaching the liver is degraded immediately[19, 29, 30]. These reports mean that the activation of FT and the inactivation of its metabolite, 5-FU, occur simultaneously in the liver. When FT was incubated with a rat liver homogenate, a little 5-FU was detected, but on adding uracil, the concentration of 5-FU increased in direct proportion to the concentration of uracil, with no change in the extent of conversion of FT to 5-FU. Namely, the degradation of 5-FU formed from FT was inhibited by uracil (Table 1). This finding may explain the increased level of 5-FU in the blood and the resulting increase of 5-FU in the tumor after coadministration of uracil with FT. KOSHIZAKA[31] reported that antimetabolites, such as 5-FU, enter the metabolic pool in the body, and especially the liver, and then are metabolized. Therefore, if the dose of FT is not sufficient to cause a change in the size of this pool, 5-FU will scarcely be released from the hepatic pool. So, for maintaining a high level of antimetabolites in the blood, it is important to fill up the pool with some other substance. From this view point, uracil may be useful in UFT therapy.

5-FU was taken up to a greater extent than uracil by AH 130 cells (Figs. 5 and 6). A similar findings in EHRLICH ascites cells was reported by HARVERS *et al.*[23] 5-FU was converted to acid-soluble F-nucleotides, and these were incorporated into RNA, but not DNA, as reported by CHAUDHURI *et al.*[21] This anabolic conversion of 5-FU was also signicantly more than that of uracil, and was scarcely affected by the presence of uracil at 100-fold the concentration as 5-FU (Table 2). FUJII *et al.*[32] reported that Km value of dihydrouracil dehydrogenase was apparently lower than those of the phosphorylating enzymes,. The Km and V_{max} values for dehydrogenation of 5-FU and uracil were 4.0×10^{-5}M and 0.51, and 1.0×10^{-5}M and 0.22, respectively; the K_m and V_{max} values for phosphorylation of 5-FU and uracil were 5.6×10^{-5}M and 1.2, and 4.5×10^{-4}M and 10 (with Rib-1-P and ATP), and 6.3×10^{-4}M and 10, and 9.1×10^{-3}M and 26 (with PPRibP), respectively. These data explain our observations described above.

5-FU inhibits the incorporation of ^{14}C-formate into the methyl group of DNA-thymine in tumor cells, and this results in enhanced utilization of exogenous thymidine[24]. After preincubation of an AH 130 cell suspension with 1×10^{-5}M and 1×10^{-4}M of 5-FU, the conversion of ^{14}C-formate to DNA-thymine was inhibited about 50% and 90%, respectively (Fig. 7); the utilization of ^{3}K-thymidine increased inversely with the concentration of 5-FU io about 2 and 3 times the control value, respectively, at the above concentrations of 5-FU (Fig. 8). These processes occurred with 5-FU whether uracil was present or absent (Tables 3 and 4).

Possible mechanisms accounting for the cytotoxicity of 5-FU include inhibition of the formation of thymidylate, blocking DNA synthesis; synthesis of malfunctioning RNA due to replacement of uracil by 5-FU; and inhibition of pyrimidine nucleotide synthesis, with resultant interference in production of RNA or DNA, or both[20]. As described above, uracil did not inhibit the anabolism of 5-FU or its cytotoxicity, even when added at 10~100 times the concentration of 5-FU. Actually, FUJII *et al.*[9], observed that uracil did not reverse the inhibitory effect of 5-FU on growth of cultured *FM3A/B* and *HeLa* cells, and similar results were reported by RICH *et al.*[13] and by REEVES *et al.*[14]

The findings obtained in the present study indicate a possible mechanism accounting for the enhanced antitumor activity of FT on coadministration of uracil in UFT therapy.

ACKNOWLEDGMENT

We thank Professor SETSURO FUJII, Division of Regulation of Macromolecular Function, Institute for Protein Research, Osaka University, for helpful advice and discussion.

REFERENCES

1) HILLER, S. A.; R. A. ZHUK & M. Y. LIDAK: Analogs of pyrimidine nucleosides. 1. N_1-(α-tetrahydrofuryl) derivatives of natural pyrimidine bases and their antimetabolites. Dokl Akad Nauk SSSR 176 : 332~335, 1967

2) FUJII, S.; K. IKENAKA, M. FUKUSHIMA & T. SHIRASAKA : Effect of uracil and its derivatives on antitumor activity of 5-fluorouracil and 1-(2-tetrahydrofuryl)-5-fluorouracil. Gann 69 : 763~772, 1978

3) FUJII, S.; S. KITANO, K. IKENAKA & T. SHIRASAKA : Studies on coadministration of uracil or cytosine on antitumor activity of FT-207 or 5-FU derivatives. Gan to Kagakuryoho 6 : 377~384, 1979

4) FUJII, S.; S. KITANO, K. IKENAKA & T. SHIRASAKA : Effect of coadministration of uracil or cytosine on the antitumor activity of clinical

doses of 1-(2-tetrahydrofuryl)-5-fluorouracil and level of 5-fluororuacil in rodents. Gann 70 : 209～214, 1979

5) TAGUCHI, T.; Y. NAKANO, K. JIKUYA, S. FUJII, K. IKENAKA, M. FUKUSHIMA & T. SHIRASAKA : Effect of uracil on the antitumor activity of Futraful. Gan to Kagakuryoho 5 : 1161～1163, 1978

6) TAGUCHI, T.; Y. NAKANO, S. FUJII & T. SHIRASAKA : Determination of 5-fluorouracil levels in tumors, blood, and other tissues. Gan to Kagakuryoho 5 : 1167～1172, 1978

7) TAGUCHI, T. & Y. NAKANO : Attainability of anticancer drugs into the tumor —UFT therapy—. Gan to Kagakuryoho 6 : 257～263, 1979

8) KIMURA, K.; Y. ISOBE, S. SUDA, Y. SUZUKI, C. KONDA, M. SHIMOYAMA, T. SAKANO & K. INOUE : Enhancement of anticancer effects by change of drug formulation, with special reference to oil bleomycin, intrarectal use of FT-207, and combination of uracil and FT-207. Gan to Kagakuryoho 6 : 237～243, 1979

9) UNEMI, N.; S. TAKEDA, K. TAJIMA, Y. KAWAGUCHI & A. YASUDA : Studies on combination therapy with 1-(2-terahydrofuryl)-5-fluorouracil plus uracil. 1. Effect of coadministration of uracil on the antitumor activity of 1-(2-tetrahydrofuryl)-5-fluorouracil and the level of 5-fluorouracil in AH 130 bearing rats. Chemotherapy 29 : 164～175, 1981

10) KAWAGUCHI, Y.; S. TAKEDA, S. NAGAYAMA, K. TAJIMA, H. MASUDA & N. UNEMI : Studies on the antitumor activity and metabolism of UFT. 11 th International Congress of Chemotherapy and 19 th Interscience Conference on Antimicrobial Agents and Chemotherapy No. 290, American Society for Microbiology, Boston, Massachusetts, U. S. A., 1979

11) TOIDE, H.; H. AKIYOSHI, Y. MINATO, H. OKUDA & S. FUJII : Comparative studies on the metabolism of 2-(tetrahydrofuryl)-5-fluorouracil and 5-fluorouracil. Gann 68 : 553～560, 1977

12) SAUNDERS, P. P. & L. Y. CHAO : Comparison of the actions of 5-fluorouracil and Ftorafur in *Escherichia coli*. Antimicr. Agents & Chemoth. 11 : 451～454, 1977

13) RICH, M. A.; J. L. BOLAFFI, J. E. KNOLL, L. CHEONG & M. L. EDINOFF : Growth inhibition of a human tumor cell strain by 5-fluorouracil, 5-fluorouridine, and 5-fluoro-2'-deoxyuridine—Reversal studies—. Cancer Res. 18 : 730～735, 1958

14) REEVES, W. J. & R. CAILLEAU : Mechanism of growth inhibition by 5-fluorouracil. Reversal studies with pyrimidine metabolites *in vitro*. Proc. Soc. Exp. Biol. & Med. 131 : 1068～1072, 1969

15) WILSON, T. H. & G. WISEMAN : The use of sacs of everted small intestine for the study of the transference of substances from the mucosal to the serosal surface. J. Physiol. 123 : 116～125, 1954

16) SCHMIDT, G. & S. J. THANHAUSER : A method for the determination of deoxyribonucleic acid, ribonucleic acid, and phosphoproteins in animal tissues. J. Biol. Chem. 161 : 83～89, 1945

17) HERSHEY, A. D.; J. DIXON & M. CHASE : Nucleic acid economy in bacteria infected with becteriophage T 2. 1. Purine and pyrimidine composition. J. Gen. Physiol. 36 : 777, 1953

18) BURTON, K.; Determination of DNA concentration with diphenylamine. in Method of Enzymology (Edited by L. GROSSMAN and K. MOLDAVE) Vol. 12, Part B, p. 163, Academic Press, New York & London, 1968

19) CHAUDHURI, N. K.; K. L. MUKHERJEE & C. HEIDELBERGER : Studies on fluorinated pyrimidines. VII. The degradative pathway. Biochem. Pharmacol. 1 : 328～341, 1959

20) MUKHERJEE, K. L. & C. HEIDELBERGER : Studies on fluorinated pyrimidines. IX. The degradation of 5-fluorouracil-6-C^{14}. J. Biol. Chem. 235 : 433～437, 1960

21) CHAUDHURI, N. K.; B. J. MONTAG & C. HEIDELBERGER : Studies on fluorinated pyrimidines. III. The metabolism of 5-fluorouracil-2-C^{14} and 5-fluoroorotic-2-C^{14} acid *in vivo*. Cancer Res. 18 : 318～328, 1958

22) BOSCH, L.; E. HARVERS & C. HEIDELBERGER : Studies on fluorinated pyrimidines. V. Effects on nucleic acid metabolism *in vitro*. Cancer Res. 18 : 335～343, 1958

23) HARVERS, E.; N. K. CHAUDHURI & C. HEIDELBERGER : Studies on fluorinated pyrimidines. VIII. Further biochemical and metabolic investigations. J. Biol. Chem. 234 : 1255～1262, 1959

24) DANNEBERG, P. B.; B. J. MONTAG & C. HEIDELBERGER : Studies on fluorinated pyrimidines. IV. Effects on nucleic acid metabolism *in vivo*. Cancer Res. 18 : 329～334, 1958

25) FUJII, S.; H. OKUDA, H. TOIDE, N. WATANABE & K. TAIRA : Studies on the fate of 1-(2-tetrahydrofuryl)-5-fluorouracil (FT-207), an antitumor agent (1) Absorption, tissue distribution and excretion. Oyo Yakuri 8 : 589～595, 1974

26) SCHANKER, L. S. & D. J. JOCCO : Some characteristics of the pyrimidine transport process

of the small intestine. Biochim. Biophys. Acta 56 : 460～469, 1962

27) SHIMOYAMA, T. *et al.*: Absorption of 1-(2-tetrahydrofuryl)-5-(fluorouracil (FT-207) from the intestine. Gan to Kagakuryoho 4 : 347～356, 1977

28) OHIRA, S.; S. MAESAWA, Y. IRINODA, K. WATANABE, K. KITADA, M. SAKUMA & T. SAITO : Studies on the cytochrome P-450 difference spectra induced by various anticancer drugs by liver microsomes from rats. Gan to Kagakuryoho 3 : 663～669, 1976

29) ANADA, S.; N. NAKAMURA & H. MARUMO : Studies on the metabolic facte of 5-fluorouracil in the oral administration. Yakugaku Zasshi 94 : 1131～1136, 1974

30) FUJIMOTO, S. *et al.*: Fundamental studies on oral administration of 5-fluorouracil. Jap. J. Cancer Clin. 22 : 28～31, 1976

31) KOSHIZUKA, K.: Fundamental studies on the hepatic metabolism of fluorinated pyrimidines. Jap. J. Cancer Clin. 23 : 916～919, 1977

32) IKENAKA, K.; T. SHIRASAKA, S. KITANO & S. FUJII : Effect of uracil on metabolism of 5-fluorouracil *in vitro*. Gann 70 : 353～359, 1979

33) SADEE, W. & C. G. WONG : Pharmacokinetics of 5-fluorouracil. Inter-relationship with biochemical kinetics in monitoring therapy. Clinical Pharmacokinetics 2 : 437～450, 1977