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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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 \(^5\)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 \(^5\)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 \(^5\)H-uracil. \(^3\)H-5-FU was rigidly 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 14C-formate into DNA-thymine and the resulting utilisation 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.\(^2\) found that codadministration 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.\(^6\) and KIMURA et al.\(^7\) 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\(^8\). After oral administration of UFT, in which FT and uracil were both rapidly 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 administration. Higher levels of 5-FU, 5-fluorouridine (FU) and 5-fluoronucleotides (F-nucleotides) were observed in the tumor than in normal tissues\(^9\).

For antitumor activity FT must be converted to its active metabolite, 5-FU\(^10\). SAUNDERS et al.\(^11\) observed that uracil reversed the growth inhibition of Escherichia coli B\(_2\) by FT and 5-FU. On the other hand, RICH et al.\(^12\) 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.\(^13\) 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.\(^12\).
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-6-3H (3H-FT, specific activity 27.8 mCi/mmole) was obtained from the Japan Radioisotope Association, Tokyo, Japan. 5-FU-6-8H (3H-5-FU, 2.0 Ci/mmole) and sodium formate-14C (14C-formate, 60.3 mCi/mmole) were purchased from the Radiochemical Centre, Amersham, England. Uracil-6-3H (3H-uracil, 23.3 Ci/mmole) and thymidine-6-8H (8H-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. Sacs of everted intestine 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 3H-FT with or without uracil, and 3H-uracil with or without FT in 100-ml beakers. The beakers were incubated at 37°C 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 days 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 but allowed free access to water. Then they were killed by decapitation and the upper part of the small intestine was removed. Sacs of everted intestine 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 3H-FT with or without uracil, and 3H-uracil with or without FT in 100-ml beakers. The beakers were incubated at 37°C 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.

**Assays on intact cells**: Freshly prepared AH 130 cells were suspended at 1×10^7 cells/ml in EAGLE's minimum essential medium (MEM) containing 10% calf serum and preincubated at 37°C for 1 hr. Then labeled precursor with or without uracil was added, and the cells were incubated for various times. Then they were centrifuged 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. The protein fraction was dissolved in NCS tissue solubilizer and other fractions were neutralized with 1 N KOH. The radioactivities of these fractions were measured.

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 14C-formate into DNA in the cells, DNA was rehydrolysed by the method of HERSHEY et al. 17), and an aliquot of the hydrolysate 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 BURTON16) 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°C for 1 hr, neutralized with acetic acid, and mixed with 10 ml of toluene-system scintillator. The radioactivity 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 method15). When the initial concentration of 3H-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). 3H-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 3H-FT (200 μg/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 rapidly 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 conversion of FT to 5-FU11) and the degradation of 5-FU to F-UPA and F-β-Ala18,20) 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 3H-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 ow 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 \(^{3}\text{H}-\text{FT}\) in a rat liver homogenate

<table>
<thead>
<tr>
<th></th>
<th>FT</th>
<th>5-FU</th>
<th>F-UPA</th>
<th>F-(\beta)-Ala</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{3}\text{H}-\text{FT})</td>
<td>467.8</td>
<td>1.3</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td>(^{3}\text{H}-\text{FT})+uracil (1 : 1)</td>
<td>458.8</td>
<td>5.1</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>(1 : 2)</td>
<td>459.7</td>
<td>6.9</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>(1 : 3)</td>
<td>472.6</td>
<td>8.6</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>(1 : 4)</td>
<td>468.8</td>
<td>9.6</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>(1 : 5)</td>
<td>470.9</td>
<td>9.3</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>(1 : 10)</td>
<td>473.4</td>
<td>11.2</td>
<td>1.8</td>
<td></td>
</tr>
</tbody>
</table>

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

Effect of uracil on the metabolism 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 \(\mu\text{g/ml}\) and 13.1 \(\mu\text{g/ml}\), 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 \(\times\) 10\(^7\) cells/ml) were incubated with \(^{3}\text{H}-\text{5-FU}\) (0.1 \(\mu\text{Ci}/\text{ml}\)) or \(^{3}\text{H}-\text{uracil}\) (0.1~10 \(\mu\text{Ci}/\text{ml}\)) 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 radioactivity was measured. The incorporations of \(^{3}\text{H}-\text{5-FU}\) into the cells in the presence of uracil are shown in Fig. 3. During incubation, \(^{3}\text{H}-\text{5-FU}\) was rapidly taken up and the total radioactivity in the cells after 60 min was about twice that after 15 min. The uptake of \(^{3}\text{H}-\text{5-FU}\) was not affected by the presence of uracil even at 100 times the concentration as \(^{3}\text{H}-\text{5-FU}\). The incorporation of \(^{3}\text{H}-\text{uracil}\) was similar to that of \(^{3}\text{H}-\text{5-FU}\) 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 showed the distribution of radioactivity in the cells after incubation of a cell suspension (1 \(\times\) 10\(^7\) cells/ml) with \(^{3}\text{H}-\text{5-FU}\) (5 \(\mu\text{Ci}/\text{ml}\)) for 1 hr. About 70% of the radioactivity taken up into the cells was present in the acid-soluble fraction, and of this radioactivity 20% was present in 5-FU, 5% in FUR and 75% in F-nucleotides. Scarcey 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 \(^{3}\text{H}-\text{FT}\) 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 \(^{3}\text{H}-\text{5-FU}\) 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 \(^{3}\text{H}-\text{5-FU}\) in...
Table 2 Effect of uracil on the anabolism of \(^{3}H\)-5-FU in intact AH 130 cells

<table>
<thead>
<tr>
<th></th>
<th>(^{3}H)-5-FU alone</th>
<th>(^{3}H)-5-FU + uracil (molar ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1 : 1)</td>
<td>(1 : 10)</td>
</tr>
<tr>
<td>Inside cells</td>
<td>381.6</td>
<td>348.6</td>
</tr>
<tr>
<td>Acid-soluble fraction</td>
<td>58.5</td>
<td>54.2</td>
</tr>
<tr>
<td>5-FU</td>
<td>13.7</td>
<td>17.6</td>
</tr>
<tr>
<td>FUrD</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F-nucleotides</td>
<td>228.4</td>
<td>194.5</td>
</tr>
<tr>
<td>Acid-insoluble fraction</td>
<td>(\text{N.E.})</td>
<td></td>
</tr>
<tr>
<td>Lipid</td>
<td>6.5</td>
<td>5.0</td>
</tr>
<tr>
<td>RNA</td>
<td>101.4</td>
<td>97.2</td>
</tr>
<tr>
<td>DNA</td>
<td>1.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Protein</td>
<td>2.7</td>
<td>2.2</td>
</tr>
</tbody>
</table>

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

The 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 \(^{23}\) and in vivo \(^{24}\). Moreover, 5-FU stimulated the incorporation of \(^{3}H\)-thymidine into DNA-thymine in all tissues tested \(^{25}\). 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/\(\mu\)g 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/\(\mu\)g (ca. 50% of control) with 1 \(\times 10^{-5}\)M and 222 dpm/\(\mu\)g (ca. 10% of control) with 1 \(\times 10^{-4}\)M. In the presence of uracil at 1 \(\times 10^{-5} \sim 1 \times 10^{-3}\)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/\(\mu\)g of DNA thymine

<table>
<thead>
<tr>
<th></th>
<th>10 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>439 (100)*</td>
<td>1,250 (100)*</td>
<td>2,448 (100)*</td>
</tr>
<tr>
<td>5-FU ((1 \times 10^{-5})M)</td>
<td>341 (78)</td>
<td>535 (43)</td>
<td>1,165 (40)</td>
</tr>
<tr>
<td>5-FU + uracil ((1 : 1))</td>
<td>193 (44)</td>
<td>600 (48)</td>
<td>1,367 (35)</td>
</tr>
<tr>
<td></td>
<td>284 (65)</td>
<td>593 (47)</td>
<td>1,090 (45)</td>
</tr>
<tr>
<td></td>
<td>308 (70)</td>
<td>895 (71)</td>
<td>1,971 (45)</td>
</tr>
<tr>
<td>uracil ((1 \times 10^{-4})M)</td>
<td>515 (117)</td>
<td>1,004 (84)</td>
<td>1,971 (40)</td>
</tr>
</tbody>
</table>

* per cent of control

\(^{14}C\)-formate: 1.0 \(\mu\)Ci, 5-FU: 1 \(\times 10^{-5}\)M, uracil: 1 \(\times 10^{-5} \sim 1 \times 10^{-4}\)M
AH 130 cells: 1 \(\times 10^7\) cells/ml of EAGLE's MEM containing 10% calf serum
Fig. 7 Effects of 5-FU and uracil on incorporation of 14C-formate into DNA thymine in AH 130 cells
AH 130 cells (1 x 10^7 cells/ml) were preincubated with 5-FU or uracil for 60 min, and then incubated with 14C-formate (60.3 mCi/m mole) at 1.0 µCi/ml for 30 min.

Fig. 8 Effects of 5-FU and uracil on incorporation of 3H-thymidine into DNA in AH 130 cells
AH 130 cells (1 x 10^7 cells/ml) were preincubated with 5-FU or uracil for 60 min, and then incubated with 3H-thymidine (23.0 Ci/m mole) at 1.0 µCi/ml for 30 min.

incorporation of 14C-formate into DNA-thymine by 1 x 10^{-5}M of 5-FU was scarcely affected (Table 3).

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

<table>
<thead>
<tr>
<th></th>
<th>10 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>99 (100)*</td>
<td>541 (100)*</td>
<td>1,220 (100)*</td>
</tr>
<tr>
<td>5-FU (1 x 10^{-8}M)</td>
<td>118 (114)</td>
<td>732 (135)</td>
<td>1,546 (127)</td>
</tr>
<tr>
<td>5-FU+uracil (1 : 1)</td>
<td>128 (130)</td>
<td>659 (122)</td>
<td>1,721 (141)</td>
</tr>
<tr>
<td>(1 : 10)</td>
<td>117 (118)</td>
<td>706 (131)</td>
<td>1,576 (128)</td>
</tr>
<tr>
<td>(1 : 100)</td>
<td>124 (126)</td>
<td>737 (136)</td>
<td>1,572 (129)</td>
</tr>
<tr>
<td>uracil (1 x 10^{-4}M)</td>
<td>100 (101)</td>
<td>510 (94)</td>
<td>1,174 (96)</td>
</tr>
</tbody>
</table>

* per cent of control

3H-thymidine : 1.0 µCi, 5-FU : 1 x 10^{-8}M, uracil : 1 x 10^{-8}~1 x 10^{-4}M
AH 130 cells : 1 x 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 blood reaches a peak within 1 hr. Uracil is absorbed by an active transport system. 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. 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 diffusion (Figs. 1 and 2).

Previously, Fujii et al. found that FT is converted to 5-FU in the presence of NAPDH, mainly by the microsomal fraction of rat liver, and they suggested that microsomal electron transport is concerned with this reaction. Ohira et al. 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. 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\(^{10,20,30}\). 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 6-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 finding in EHRlich ascites cells was reported by HARVERS et al.\(^{119}\). 5-FU was converted to acid-soluble F-nucleotides, and these were incorporated into RNA, but not DNA, as reported by CHAUDHURI et al.\(^{21}\). Anabolic conversion of 5-FU was also significantly more than that of uracil, and was scarcely affected by the presence of uracil at 100-fold the concentration of 5-FU (Table 2). FUJI et al.\(^{32}\) reported that Km value of dihydouracil dehydrogenase was apparently lower than those of the phosphorylating enzymes. The Km and \(V_{\text{max}}\) values for dehydrogenation of 5-FU and uracil were 4.0 \(\times 10^{-9}\)M and 0.51, and 1.0 \(\times 10^{-9}\)M and 0.22, respectively; the Km and \(V_{\text{max}}\) values for phosphorylation of 5-FU and uracil were 5.6 \(\times 10^{-9}\)M and 1.2, and 4.5 \(\times 10^{-9}\)M and 10 (with Rib-1-P and ATP), and 6.3 \(\times 10^{-9}\)M and 10, and 9.1 \(\times 10^{-9}\)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\(^{40}\). After preincubation of an AH 130 cell suspension with 1 \(\times 10^{-10}\)M and 1 \(\times 10^{-9}\)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 5-FU-thymidine increased inversely with the concentration of 5-FU to 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\(^{40}\). 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, FUJI et al. 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.\(^{12}\) 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.

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