Inhibition by Oxonic Acid of Gastrointestinal Toxicity of 5-Fluourouracil without Loss of Its Antitumor Activity in Rats

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ABSTRACT

The possibility of decreasing the gastrointestinal (GI) toxic effects of 5-fluourouracil (5-FU) on the digestive tract such as its injury of cells and induction of diarrhea, without reducing its antitumor activity, was investigated in rats. Oxonic acid was found to inhibit the phosphorylation of 5-FU to 5-fluourouridine-5'-monophosphate catalyzed by pyrimidine phosphoribosyltransferase in a different manner from allopurinol in cell-free extracts and intact cells in vitro.

On p.o. administration of 5-FU (2 mg/kg) and a potent inhibitor of 5-FU degradation to Yoshida sarcoma-bearing rats, oxonic acid (10 mg/kg) was found to inhibit the formation of 5-fluourouridine-5'-monophosphate from 5-FU and its subsequent incorporation into the RNA fractions of small and large intestine but not of tumor and bone marrow tissues. This selective inhibition of 5-FU phosphorylation in the GI tract was due to the much higher concentrations of oxonic acid in GI tissues than in other tissues and the blood. On p.o. administration with the 5-FU derivative, UFT, which is a combined form of tegafur and uracil, oxonic acid markedly reduced injury of GI tissues and/or severe diarrhea without influencing the antitumor effect of UFT.

These findings suggest that coadministration of oxonic acid suppresses the GI toxicity of 5-FU and its derivatives without affecting their antitumor activity and thus prolongs the life span of cancer-bearing rats.

INTRODUCTION

5-FU2 has been used extensively in treatment of patients with cancer of the breast, stomach, colorectum, head and neck, and gynecological tracts. However, low response rates (about 10 to 20%) to i.v. bolus doses of 5-FU and a short durations of remission have been observed. This low response to 5-FU is thought to be due to side effects, such as the GI, myelotoxicities, neurotoxicities, and cardiotoxicities induced by 5-FU, which prevent its administration for a long periods and/or at high doses, and its rapid catabolism in the body before it can exert a significant antitumor effect.

Recently, CVI of 5-FU has been found to increase the response rates of patients with gastric, colorectal or breast cancers to about 30 to 60% (1–7). Rokich et al. (8) also reported that long-term CVI of 5-FU at 300 mg/m2/day resulted in a higher response rate than its i.v. bolus administration at 500 mg/m2 in a randomized study on adjuvant chemotherapy of metastatic colorectal cancers. However, GI toxicity, but not myelosuppression, is a dose-limiting factor during CVI of 5-FU. Caballero et al. (1) also reported no myelosuppression, alopecia, nausea, or vomiting during long-term CVI of 5-FU for 54 to 324 days. These findings indicate that suppression of the GI toxicity of 5-FU would result in a much higher response rate on infusion therapy with 5-FU. There are reports that the GI toxicity and myelotoxicity of 5-FU are caused by its phosphorylation in the digestive tract (9) and bone marrow tissue (10), whereas its cardiotoxicity and neurotoxicity are induced by its catabolic products in the heart and brain (11).

Schwartz and Handschmacher (12) reported that allopurinol, a potent inhibitor of xanthine oxidase, antagonized the growth-inhibitory effects of 5-FU on various murine leukemia cell lines, but not on Walker 256 or Hela cell lines in vitro. They also demonstrated that allopurinol reduced the lethal toxicity of a high dose 5-FU and delayed tumor growth 100% more than the optimal administration schedule of 5-FU in colon 38-bearing mice (13). Later, Galewal et al. (14) found that oxipurinol, a metabolite of allopurinol, did not inhibit the cytotoxicity of 5-FU on human tumor cell lines. Based on these in vitro and in vivo studies, clinical trials to reduce the toxicity of 5-FU and increase the response rate were carried out using a combination of CVI or bolus 5-FU (2 to 2.5 g/m2/day) and p.o. allopurinol (900 mg/body). However, neither decreased toxicity nor an increased response rate was observed (15–19).

This prompted us to search for an inhibitor of 5-FU phosphorylation, catalyzed by pyrimidine phosphoribosyltransferase (EC 2.4.2.10), for preventing GI toxicity in tumor-bearing rats during 5-FU therapy. We found that oxonic acid (5-azaotic acid) strongly inhibited the phosphorylation of 5-FU to FUMP both in vitro and in vivo. The present article reports inhibition of 5-FU-induced GI toxicity without loss of its antitumor activity by its coadministration with oxonic acid in tumor-bearing rats.

MATERIALS AND METHODS

Chemicals. 5-FU was purchased from Sigma Chemical Co., St. Louis, MO. Oxonic acid was obtained from Aldrich Chemical Co., USA. [6-'H]5-FU (710.4 GBq/mmol) was from New England Nuclear. [2,4-14C]Potassium oxonate (851 MBq/mmol) was from Amersham Co. 5-FU derivatives, UFT (20, 21), were products of Taiho Pharmaceutical Co., Tokyo, Japan. CNDP, potent inhibitors of 5-FU degradation (22), were from Otsuka Pharmaceutical Co., Tokyo, Japan. All other chemicals used were standard commercial products.

Tumor Cells and Antitumor Experiments. Yoshida sarcoma cells were supplied by Sasaki Research Institute, Tokyo, Japan, and passaged in male Donryu strain rats weighing about 150 g by i.p. inoculation at weekly intervals. Groups of 8 rats were used. Solid-type Yoshida sarcoma was prepared by s.c. transplantation of 2 × 103 cells into the back of rats on day 0. Drugs, suspended in 1% HPMC solution, were administered p.o. daily for 7 consecutive days, starting 24 h after implantation of tumor cells. Control rats were given 1% HPMC solution only by the same schedule. On day 8, the rats were killed and their tumors were removed and weighed for evaluation of antitumor activity (inhibition of tumor growth).

Pathological Evaluation of Injury of the Digestive Tract. After the above therapeutic experiments, most of the digestive tract was removed, washed with saline, and soaked in 10% formaldehyde solution. Then, the duodenum, jejunum, ileum, cecum, colon, and rectum were separated, and specimens for pathological observation were prepared by standard methods. After staining with hematoxylin-eosin, degrees of injury such as bleeding, necrosis, glandular expansion, decrease in glandular tubes, loss of cilia, and a degenerative appearance were scored under a light microscope as ( ) none, ( + ) slight, ( ++ ) medium, and ( + + + ) severe. The incidence of GI toxicity was expressed as the number of rats with grade ( + ) to ( + + + ) GI injury in groups of 8 rats.

Assay of 5-FU Phosphorylation. The enzyme was prepared from Yoshida sarcoma cells. Tumor cell pellets were homogenized with 4 volumes of 50 mm Tris-HCl (pH 8.0) containing 5 mm 2-mercaptoethanol. The homogenate was frozen 2(9)/93; accepted 8/23/93.

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1 To whom requests for reprints should be addressed.
2 The abbreviation used are: 5-FU, 5-fluorouracil; FUMP, 5-fluorouridine 5'-monophosphate; GI, gastrointestinal; FT, tegafur; CVI, continuous venous infusion; CNDP, 3-cyano-2,6- dihydroxypyridine; HPMC, hydroxpropylmethyl cellulose; TCA, trichloroacetic acid; IC50, 50% inhibitory concentration; UFT, 1 m tegafur-4 m uracil; Cmax, maximum concentration.

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centrifuged at 105,000 g for 60 min, and the supernatant fraction was used for assay of 5-FU phosphorylation as described before (23). The phosphorylation of 5-FU to FUMP catalyzed by pyrimidine phosphoribosyltransferase (EC 2.4.2.10) and its inhibition by oxonic acid and other compounds were studied in incubation mixture containing 50 mM Tris-HCl (pH 8.0), 50 mM MgCl2, 10 mM NaF, 4 mM phosphopropylphosphate, 10 mM [6-3H]-5-FU (74 kBq), 50 μM of inhibitor solution, and 150 μl of enzyme solution in a final volume of 0.25 ml. The incubation was performed at 37°C for 30 min and was terminated by the addition of 2 ml perchloric acid (50 μl) followed by centrifugation at 3000 rpm for 10 min. Then 100 μl of the supernatant were neutralized with 30 μl of 2 M KOH solution and 20 μl aliquots were subjected to silica gel 60F254 thin layer chromatography with a mixture of chloroform, methanol, and acetic acid (17:3:1, v/v/v) as the mobile phase. Spots of FUMP were scraped into vials and extracted with 0.1 ml of 4 M HC1. The extracts were mixed with 10 ml of scintillation fluid (ACS-II; Amershain) and radioactivity was measured in a liquid scintillation spectrometer. This assay system was used to determine IC50 values.

**Phosphorylation of 5-FU in Intact Cells in Vitro.** Samples of about 2 × 107 ascitic Yoshida sarcoma or intact rat bone marrow cells suspended in Eagle’s minimum essential medium were incubated with 1 μM [6-3H]-5-FU (57 kBq) alone or in the presence of inhibitors in a final volume of 2 ml at 37°C for 10 min and then 2 ml of 10% TCA were added. The mixtures were centrifuged at 2000 rpm for 5 min, and then the TCA-soluble fraction was neutralized with KOH and 50-μl aliquots were subjected to silica gel thin layer chromatography as described above. The spot of FUMP was scraped off for measurement of its radioactivity.

**Phosphorylation of 5-FU and Its Subsequent Incorporation into RNA of Tissues of Tumor-bearing Rats.** [6-3H]-5-FU (2 mg/kg; 7.4 MBq/kg) in combination with a 10-fold m excess of CNDP, a potent inhibitor of 5-FU degradation (22), was administered with or without oxonic acid to Yoshida sarcoma-bearing rats on day 8 after tumor implantation. Three h later, the rats were sacrificed and their tumor, small and large intestine, and bone marrow tissues were removed and promptly frozen and kept at −80°C until use. The tissues were homogenized with 3 volumes of 10% TCA and centrifuged at 3000 rpm for 10 min. The resultant supernatant neutralized by shaking with an equal volume of 17.7% (v/v) tri-n-octylamine in 1,1,2-trichloro-1,2,2-trifluoroethane and then evaporated under vacuum. The residue was dissolved in distilled water and passed through a 0.45-μm filter, and the radioactive FUMP in the filtrate was separated by high performance liquid chromatography. An aliquot of the sample was chromatographed on a column (4.6 ID X 250 mm) from Nargel Chemicals, Germany, under the following chromatographic conditions: flow rate, 1 ml/min; mobile phase, 5% methanol containing 2 mM tetraethyl ammonium hydrogen sulfite (pH 2.5). The eluate was collected in a microfraction collector and the radioactivity of FUMP was counted. The radioactivity incorporated into RNA present in the TCA-precipitated material was extracted by the method of Schneider (24) for determination of the amount of 5-FU incorporated into RNA.

**Determination of [2,4-14C]Oxonic Acid and Its Catabolites in Serum and Tissues of Tumor-bearing Rats.** 14C-oxonic acid (10 mg/kg; 37 MBq/kg) dissolved in 1% HPMC solution was administered p.o. to Yoshida sarcoma-bearing rats. The animals were then sacrificed at intervals and their blood, tumors, small and large intestine, liver, and kidney were removed and promptly frozen at −30°C. For examination, the tissues were homogenized in 4 volumes of 10 mM Tris-HCl (pH 7.4). These homogenates and the serum were heated for 3 min in a boiling water bath and then cooled in an ice-bath and centrifuged at 3000 rpm. The resulting supernatants were mixed with an equal volume of ethanol, stood for 20 min, and centrifuged. The clear supernatants were evaporated under vacuum at 40°C, and the residues were dissolved in water. Aliquots were then analyzed by high performance liquid chromatography on a Nucleosil-SC18 column (inside diameter, 4.6 × 250 mm) from Nargel Chemicals, Germany, under the following conditions: flow rate, 1 ml/min; mobile phase, 5% methanol containing 2 mM tetrabutyl ammonium hydrogen sulfite (pH 2.5). The eluate was monitored with a 14C-radioanalysis detector. In this condition, 14C-oxonic acid and its metabolites containing 14C-5-azauracil were eluted at the retention time of 11.0 and 4.2 min, respectively. Throughout these procedures, control 14C-oxonic acid was not almost degraded and about 95.2% of it was recovered. The portions of oxonic acid and its catabolites were pooled, respectively, and those radioactivities were measured. The contents of 14C-oxonic acid and its catabolites in serum and tissues were calculated from the specific radioactivity of 14C-oxonic acid sample (0.850 MBq/μmol).

**RESULTS**

**Effects of Oxonic Acid and Other Compounds on 5-FU Phosphorylation in Vitro.** The inhibitory effects of commercially available compounds on 5-FU phosphorylation were examined using enzyme extracts from Yoshida sarcoma cells. Table 1 shows the IC50 values of oxonic acid and several other compounds on the phosphorylation of 5-FU to FUMP. Oxonic acid, orotic acid, and citrazinic acid strongly inhibited the phosphorylation of 5-FU (1 μM) to FUMP catalyzed by pyrimidine (orotate) phosphoribosyltransferase (EC 2.4.2.10) with IC50 values ranging from 1 to 4 μM but had no effect on the reaction catalyzed by uracil ribosyl transferase (EC 2.4.2.3) and uridine kinase (EC 2.7.1.48). Allopurinol, a strong inhibitor of xanthine oxidase (EC 1.2.3.2), and 5-azauracil, a catabolite of oxonic acid, did not inhibit the phosphorylation of 5-FU in the conditions used. The effects of these compounds on intracellular phosphorylation of 5-FU were investigated using ascitic Yoshida sarcoma (as tumor) and intact bone marrow (as normal) cells. As shown in Fig. 1, oxonic acid and allopurinol inhibited the intracellular phosphorylation of 5-FU to FUMP in both cells lines. The inhibitory potency of oxonic acid was stronger than that of allopurinol. Other compounds tested, orotic acid, citrazinic acid, and barbituric acid, did not inhibit the intracellular 5-FU phosphorylation, suggesting low uptakes of these compounds into the cell lines examined (data not shown). These results indicate that oxonic acid caused direct inhibition of the phosphorylation of 5-FU to FUMP catalyzed by orotate phosphoribosyltransferase (EC 2.4.2.10), while an active metabolite (phosphorylated form of oxipurinol riboside) of allopurinol inhibits only the phosphorylation of 5-FU to FUMP.

**Inhibitory Effect of Oxonic Acid on in Vivo Phosphorylation of 5-FU in Rats.** When administered to rats, large portions of 5-FU are known to rapidly catabolized in the liver mainly. Therefore, the behavior of 5-FU in the body would be markedly different between rats and also affected to total body clearance of it and/or ciradian rhythm in each rat. These problems are thought to be settled by coadministration of a potent inhibitor of 5-FU degradation in the liver with 5-FU. From our previous investigation (22, 25), we basically used CNDP, a potent inhibitor of 5-FU, degradation to evaluate exactly the extent of phosphorylation of 5-FU in the experimental models. CNDP was not affected on absorption of 5-FU in digestive tracts of the rat and also phosphorylation of 5-FU and its subsequent incorporation into RNA in vivo.

5-FU (2 mg/kg; 15.4 μmol/kg) combined with a 10-fold m excess of CNDP and oxonic acid (10 mg/kg; 51.3 μmol/kg) were coadministered p.o. to Yoshida sarcoma-bearing rats and the FUMP contents and 5-FU incorporations into RNA of the tumor, small intestine, and bone were investigated using ascitic Yoshida sarcoma (as tumor) and intact bone marrow (as normal) cells. As shown in Fig. 1, oxonic acid and allopurinol inhibited the intracellular phosphorylation of 5-FU to FUMP in both cells lines. The inhibitory potency of oxonic acid was stronger than that of allopurinol. Other compounds tested, orotic acid, citrazinic acid, and barbituric acid, did not inhibit the intracellular 5-FU phosphorylation, suggesting low uptakes of these compounds into the cell lines examined (data not shown). These results indicate that oxonic acid caused direct inhibition of the phosphorylation of 5-FU to FUMP catalyzed by orotate phosphoribosyltransferase (EC 2.4.2.10), while an active metabolite (phosphorylated form of oxipurinol riboside) of allopurinol inhibits only the phosphorylation of 5-FU to FUMP.

| Table 1. Inhibitory effects of some pyrimidine and pyridine derivatives on 5-FU phosphorylation in Yoshida sarcoma extracts |
| --- | --- |
| Compound | IC50 value (μM) |
| | 5-FU → FUMP | 5-FU → Furdo → FUMP |
| Orotic acid | 3.70 | NI* |
| Allopurinol | 2.80 | NI |
| Citrazinic acid | 0.92 | NI |
| 5-Azauracil | 3.01 | NI |
| Barbituric acid | 0.01 | NI |
| 5-Azauracil | NI | >500 |

* NI, not inhibited.

4005
These results suggest that on p.o. administration, oxonic acid becomes located in GI tissues but not in other tissues, including tumor and bone marrow tissues, and consequently selectively inhibits the formation of FUMP from 5-FU in GI tissues. Oxonic acid is known to be catabolized to 5-azauracil and then to formyl biuret (26), so we investigated the inhibitory effect of i.p. 5-azauracil on the phosphorylation of 5-FU and its incorporation into RNA in rat tissues. As shown in Fig. 2, 5-azauracil did not inhibit both the formation of FUMP from 5-FU and its subsequent incorporation into RNA. Accordingly, the inhibition of phosphorylation of 5-FU and its subsequent incorporation into RNA is concluded to be due to oxonic acid, not 5-azauracil.

**Blood and Tissue Levels of Oxonic Acid in Tumor-bearing Rats after Its p.o. Administration.** 14C-oxonic acid (10 mg/kg; 3.7 MBq/kg) was given p.o. to Yoshida sarcoma-bearing rats and then its distribution in the blood, tumor, and normal tissues was investigated. As shown in Fig. 3, the active oxonic acid was much higher distributed in small intestine (Cmax, 54.6 nmol/g) and large intestine (Cmax, 11.8 nmol/g) and persisted longer than that in the blood (Cmax, 0.8 nmol/g), tumor (not detectable), liver (Cmax, 4.5 nmol/g), and kidney (Cmax, 3.1 nmol/g). On the other hand, the levels of an inactive metabolites derived from oxonic acid were objective in the liver (Cmax, 5.0 nmol/g), kidney (Cmax, 13.0 nmol/g), small (Cmax, 8.5 nmol/g), and large (Cmax, 10.4 nmol/g) intestine, but very low in the serum (Cmax, 2.1 nmol/g) and tumors (Cmax, 1.7 nmol/g) (data not shown). This finding is presumably closely related with the selective inhibition of 5-FU phosphorylation in the digestive tract of these rats.

**Table 2. Effects of oxonic acid and allopurinol on 5-FU phosphorylation in tissues of Yoshida sarcoma-bearing rats given [6-14H]FUMP with CNDP**

<table>
<thead>
<tr>
<th>Effector (mg/kg)</th>
<th>[6-14H]FUMP formed (nmol/g tissue ± SD)</th>
<th>Route</th>
<th>Small intestine</th>
<th>Yoshida sarcoma</th>
<th>Bone marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxonic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>p.o.</td>
<td>0.732 ± 0.006</td>
<td>0.903 ± 0.091</td>
<td>0.874 ± 0.05</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>p.o.</td>
<td>0.228 ± 0.057  (69)a</td>
<td>0.885 ± 0.125 (2)</td>
<td>0.950 (0)</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>i.p.</td>
<td>1.436 ± 0.051</td>
<td>1.018 ± 0.156</td>
<td>1.644</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>i.p.</td>
<td>0.350 ± 0.110  (76)</td>
<td>0.250 ± 0.087 (75)</td>
<td>0.594 (64)</td>
</tr>
<tr>
<td>Allopurinol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>p.o.</td>
<td>1.730 ± 0.093</td>
<td>2.188 ± 0.991</td>
<td>1.005</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>p.o.</td>
<td>1.739 ± 0.242 (0)</td>
<td>1.291 ± 0.294 (41)</td>
<td>0.726 (28)</td>
</tr>
<tr>
<td>50</td>
<td>2</td>
<td>p.o.</td>
<td>0.728 ± 0.053  (58)</td>
<td>0.873 ± 0.146 (60)</td>
<td>0.357 (64)</td>
</tr>
</tbody>
</table>

*a Combined with 10-fold w excess of CNDP.
*b Values are means ± SD for 3 rats.
*c Value in cell pellets collected from 3 rats.
*d Percent inhibition.
OXONIC ACID MODULATION OF 5-FU PHOSPHORYLATION

Table 3. Effects of oxonic acid and allopurinol on 5-FU incorporation into RNA of tissues of Yoshida sarcoma-bearing rats given [6-3H]5-FU combined with CNDP

<table>
<thead>
<tr>
<th>Effector (mg/kg)</th>
<th>[6-3H]5-FU* with CNDP (mg/kg)</th>
<th>Route</th>
<th>[6-3H]5-FU contents in RNA (nmol/g tissue ± SD)*a,b,c,d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxonic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>p.o.</td>
<td>4.31 ± 0.33</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>p.o.</td>
<td>1.27 ± 0.20 (71)</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>i.p.</td>
<td>3.98 ± 0.25</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>i.p.</td>
<td>2.08 ± 0.43</td>
</tr>
<tr>
<td>Allopurinol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>p.o.</td>
<td>3.92 ± 0.13</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>p.o.</td>
<td>3.45 ± 0.26 (12)</td>
</tr>
<tr>
<td>50</td>
<td>2</td>
<td>p.o.</td>
<td>1.82 ± 0.24 (54)</td>
</tr>
</tbody>
</table>

* Combined with 10-fold excess of CNDP.
* Values are means ± SD for 3 rats.
* Value in cell pellets collected from 3 rats.
* Percent inhibition.

DISCUSSION

Continuous infusion of 5-FU alone or in combination with a modulator such as leuokovin or cisplatin has resulted in a relatively high response rate (about 40–90%) in patients with metastatic colorectal carcinoma and squamous cell carcinoma of the head and neck. However, in this treatment severe GI toxic effects, including stomatitis and diarrhea, appeared to be dose-limiting factors. Suppression of 5-FU-induced GI toxicity should, therefore, be effective for increasing the duration of response to 5-FU and the survival of cancer patients. Allopurinol has been reported to influence the phosphorylation of 5-FU in intact tumor and bone marrow cells. Its inhibitory potency on 5-FU phosphorylation was stronger than that of allopurinol.

Oxonic acid is known to inhibit de novo pyrimidine biosynthesis (29, 30) and has been administered i.p. to cancer patients (31), although it shows no antitumor activity.

Therefore, we tested the effect of oxonic acid on the GI toxicity during 5-FU therapy. We measured the amounts of FUMP formed from 5-FU in the tumor, small intestine, and bone marrow tissues of rats after administration of oxonic acid with 5-FU. To evaluate properly the inhibitory effects of oxonic acid on the phosphorylation of 5-FU in various tissues of tumor-bearing rats in vivo studies we also administered the potent inhibitor of 5-FU degradation CNDP, which we found previously (22) inhibited the rapid degradation of 5-FU in the liver. We found that when administered p.o. with 5-FU, oxonic acid strongly inhibited the conversion of 5-FU to FUMP in the small intestine but not in Yoshida sarcoma and bone marrow tissues. We also found that after its p.o. administration oxonic acid accumulated in GI tissues more than in other tissues or the blood of tumor-bearing rats. These two findings indicate that oxonic acid, coadministered p.o. with 5-FU or a masked form of 5-FU, reduced the incidence of GI toxicity but not the antitumor effects of these compounds. In contrast, the p.o. allopurinol (50 mg/kg) in combination with 5-FU inhibited the conversion of 5-FU to FUMP in all tissues tested, suggesting that allopurinol...
OXONIC ACID MODULATION OF 5-FU PHOSPHORYLATION

Table 4. Effects of p.o. coadministration of oxonic acid on antitumor activity and GI toxicity of UFT in Yoshida sarcoma-bearing rats

<table>
<thead>
<tr>
<th>UFT (mg/kg)</th>
<th>Oxo* (mg/kg)</th>
<th>Mol. ratio Oxo/FT</th>
<th>n</th>
<th>Tumor wt. (g ± SD)</th>
<th>Diarrhea</th>
<th>Incidence of pathol. injury in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Duodenum</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Gr.-</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>16</td>
<td>1.05 ± 0.30</td>
<td>0</td>
<td>16b</td>
</tr>
<tr>
<td>50</td>
<td>---</td>
<td>0</td>
<td>8</td>
<td>0.00 ± 0.00</td>
<td>12.5</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td>0.1</td>
<td>8</td>
<td>0.00 ± 0.00</td>
<td>25.0</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>10</td>
<td>0.2</td>
<td>8</td>
<td>0.00 ± 0.00</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>20</td>
<td>0.4</td>
<td>7</td>
<td>0.00 ± 0.00</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>1.0</td>
<td>8</td>
<td>0.00 ± 0.00</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Oxonic acid.

b Number of rats in which pathological injury was observed.

Fig. 4. Light microscopic appearance of jejunum of tumor-bearing rats. A. no drugs; B. UFT alone (50 mg/kg); C. UFT plus oxonic acid (both 50 mg/kg).

In chemotherapeutic experiments on tumor-bearing rats, the high doses of 5-FU or its derivatives necessary for complete inhibition of tumor growth induced severe diarrhea and injury of the digestive tract. In the present experiments 50 mg/kg of UFT (20, 21), which almost completely inhibits tumor growth, was administered daily for 7 days. p.o. coadministration of 20 to 50 mg/kg of oxonic acid, (0.4-1 M equivalents of tegafur), markedly reduced the diarrhea and injury of the digestive tract (grade +) without appreciably affecting the antitumor activity of UFT. In adjuvant chemotherapy, prolongation of survival of patients is important as well as a higher response rate and may be attained by long-term administration of 5-FU itself or in a masked form if GI toxicity is suppressed. The combination of oxonic acid and a 5-FU derivative described here may be useful for this purpose.

Stomatitis, one sign of GI toxicity, is also a major dose-limiting factor during continuous infusion 5-FU. Clark et al. (32) reported that frequent mouthwash with allopurinol reduced the incidence of severe stomatitis in patients during 5-FU therapy, whereas Vliet et al. (33) and Loprinzi et al. (34) found that allopurinol mouthwash was ineffective against 5-FU-induced stomatitis. The concentrations of allopurinol and treatment schedules used in these clinical studies differed, so it is still uncertain whether mouthwash with allopurinol is effective against 5-FU-induced stomatitis and/or mucositis. The present results show that oxonic acid is a stronger inhibitor of the conversion of 5-FU to FUMP than allopurinol and that it is absorbed from digestive tract into the blood circulation less than allopurinol. Thus oxonic acid mouthwash should be more effective for preventing 5-FU-induced stomatitis. Further studies on the effect of oxonic acid on prolongation of the survival time of animals and protection against 5-FU-induced stomatitis are in progress.

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Inhibition by Oxonic Acid of Gastrointestinal Toxicity of 5-Fluorouracil without Loss of Its Antitumor Activity in Rats

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