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Original Article

Pharmacokinetics of Gamma-Hydroxybutylic Acid (GHB) and Gamma-Butyrolactone (GBL), the Anti-Angiogenic Metabolites of Oral Fluoropyrimidine UFT, in Patients with Gastric Cancer

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Abstract Gamma-hydroxybutylic acid (GHB) and gamma-butyrolactone (GBL), the metabolites of UFT, which is an oral fluoropyrimidine, have been reported to inhibit angiogenesis with IC50 values of 25.8 ng/ml. The pharmacokinetics of GHB and GBL were examined after the administration of UFT in patients with gastric cancer. The patients received 200 mg of UFT orally twice a day. Peripheral blood samples were collected at 0, 0.5, 1, 2 and 4 hr after the time of dosing on day 5. The baseline and endogenous GBL concentrations in plasma were 20.2 ± 7.5 ng/ml for patients and 16.8 ± 4.0 ng/ml for volunteers (P = 0.221). The values of Cmax for tegafur, uracil, 5-FU and GBL were 14.7 ± 5.2 and 4.0 ± 2.8 μg/ml, 191.2 ± 115.3 and 147.5 ± 57.3 ng/ml, respectively, and the values of Tmax were 1.0 ± 0.6, 1.1 ± 0.6, 0.9 ± 0.6 and 1.2 ± 0.6 hr, respectively. The concentration of GBL was much higher than its IC50 value for angiogenesis. GBL is thus suggested to contribute to the anticancer effects of UFT in addition to that of 5-FU, which is continuously metabolized from UFT.

Key words : gastric cancer, GBL, GHB, UFT

Introduction

UFT is an oral fluoropyrimidine formed by the combination of uracil and 1-(2-tetrahydrofuryl)-5-fluorouracil (tegafur) at a molar ratio of 4 : 1. It was developed by Fujii et al.[1)~4)] in Japan and has been used for the treatment of various solid cancers[5)] (Fig. 1). Tegafur is a prodrug that is gradually converted into fluorouracil (5- FU) in the liver by p-450 enzyme. Uracil enhances the serum concentration of 5-FU by the competitive inhibition of dihydropyrimidine dehydrogenase (DPD), which is the enzyme responsible for 5-FU catabolism[4)]. Oral UFT generates a higher maximal plasma level of 5-FU than the protracted intravenous injection of 5-FU given in a dose that is equimolar to the amount of tegafur in UFT[2)3)].

UFT is effective as adjuvant chemotherapy for gastric cancer[6)], colon cancer[7)], rectal cancer[8)], non-small-cell lung cancer[9)] and breast cancer[10)].

Recent reports have shown gamma-hydroxybutylic acid (GHB) and gamma-butyrolactone (GBL), the metabolites of tegafur (Fig. 1), in chemical equilibrium under physiological conditions, to inhibit angiogenesis in an experimental system[11)12)].

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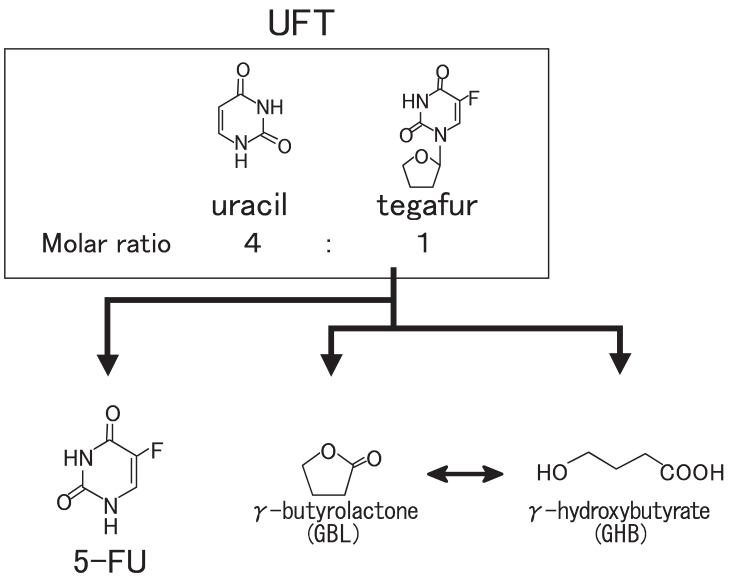

Fig. 1 UFT and its metabolites

However, the pharmacokinetics of these metabolites, has not yet been clarified yet in humans. The concentration of GBL after oral administration of UFT has not been demonstrated to obtain the level of anti-angiogenesis. Only the GBL concentration after the intravenous rapid administration of tegafur had been reported[13]. The present study examined the pharmacokinetics of GBL after the oral administration of UFT in patients with Stage I gastric cancer, in comparison to the endogenous GBL level in healthy volunteers.

Patients and methods

Patients

Ten patients, including 7 males and 3 females, with histologically identified gastric cancer and a median age of 60 years (ranging from 48 to 69 years old) and ten male healthy volunteers were enrolled in this study. Written informed consent was obtained from all the patients and volunteers who participated in this study.

Administration, blood sampling and treatment

The patients received 200 mg of UFT (tegafur-uracil ; Taiho Pharmaceutical Co., Ltd., Tokyo, Japan) twice a day. The patients fasted for 1 hr before and after administration. Four ml of heparinized peripheral venous blood was collected at 8 : 00am in the morning before the start of administration as a baseline for the respective cases. Blood was also collected from healthy volunteers as controls under the same conditions. Blood was collected at 0, 0.5, 1, 2 and 4 hr after the time of dosing at 8 : 00 in the morning on day 5 after the start of administration of UFT. The blood was centrifuged at 5°C and 3,000 r.p.m. for 10 min to separate the plasma. The plasma was immediately frozen and stored at −80°C until analysis.

Determination of drug concentrations Tegafur, 5-FU and uracil

The concentrations of tegafur and 5-FU were analyzed by the modified modification of the methods of Matsushima et al.[14], and uracil was determined according to the method of Marunaka et al.[15].

GHB and GBL

GHB and GBL were extracted with dichloromethane from the samples under acidic conditions established with hydrochloric acid according to the report of Fukui et al.[16]. GBL and GHB are the tautomers in solution as well under physiological conditions. Hydrochoric acid was added to quantitatively convert GHB into GBL, then GBL was quantified as the sum of GHB and GBL. A 0.2 ml aliquot of plasma, 100 ng of GBL-d6 (Aldrich Chemical Co., Milwaukee, WI, USA) as the internal standard and 0.5 ml of 6mol/l hydrochloric acid (Wako Pure Chemical, Osaka, Japan) were added. To this solution, 2 ml of dichloromethane (Wako Pure Chemical) was added, and the solution was then shaken for 10 min and centrifuged at 5°C and 2,500 r.p.m. This procedure was repeated twice. The dichloromethane layers were combined and concentrated at 35°C in a stream of nitrogen to 50 μl to obtain the sample for quantification. GC/MS analysis was conducted using a Trace GC-MS, quadropole mass spectrometer with gas chromatography (Thermo Fisher, San Jose, CA, USA), with a DB-Wax capillary column (J&W, 30m x 0.32 mm ID, film thickness: 0.25 μm).

Pharmacokinetic and statistical analyses

The maximum plasma concentration and the time required to reach the maximum plasma concentration were defined as Cmax and Tmax, respectively. Data are expressed as the mean with standard deviation (SD). Student's t-test was performed to identify any significant differences between the baseline GBL values for patients with gastric cancer and healthy volunteers, and a two-tailed level of $P<0.05$ was considered to be significant.

Results

The baseline GBL concentrations in plasma were 20.2 ± 7.5 ng/ml for patients

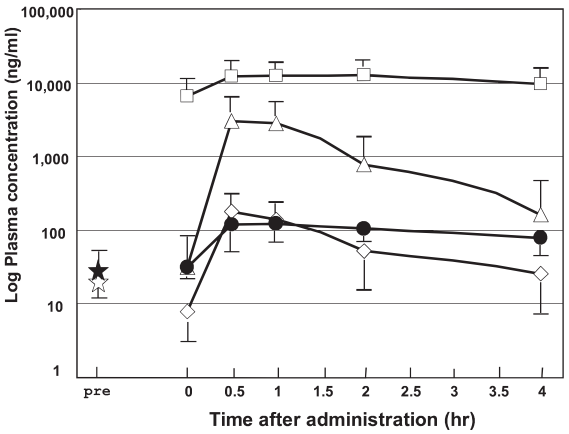

Fig. 2 Plasma concentration of UFT metabolites, Tegafur (□), 5-FU (◇), Uracil (△), GBL (●). The data points were expressed with logarithmic plots for Y axis. The data points of "pre" indicate the endogenous GBL concentrations of cancer patients (★) and healthy volunteer (☆).

Table 1 The concentration of Tegafur, 5-FU, Uracil and GBL

Patient	UFT dosage (mg/m²/day)	Tegafur (μg/ml)		Tegafur (μg/ml)		Tegafur (μg/ml)		Tegafur (μg/ml)		
		Cmax	Tmax	Cmax	Tmax	Cmax	Tmax	Base line	Cmax	Tmax
1	265.4	18.9	1.0	126.2	1.0	3.46	1.0	34.8	152.1	1.0
2	251.9	12.5	1.0	86.6	0.5	0.97	1.0	19.9	113.4	1.0
3	285.0	8.3	1.0	51.5	1.0	0.36	0.5	17.7	176.3	1.0
4	232.5	16.4	1.0	298.3	0.5	5.58	1.0	11.4	182.0	0.5
5	223.4	10.1	2.0	62.8	2.0	0.74	2.0	15.1	104.4	2.0
6	263.3	16.4	0.5	371.3	0.5	8.21	1.0	28.5	275.6	0.5
7	285.8	26.3	2.0	130.1	2.0	3.72	2.0	19.7	72.8	2.0
8	274.5	14.1	0.5	288.9	0.5	4.98	1.0	26.0	114.9	1.0
9	242.0	13.7	0.5	198.6	0.5	6.37	0.5	17.1	115.3	2.0
10	257.8	10.7	0.5	297.6	0.5	6.07	0.5	11.8	167.8	0.5
Mean	258.2	14.7	1.0	191.2	0.9	4.0	1.1	20.2	147.5	1.2
SD	21.1	5.2	0.6	115.3	0.6	2.7	0.6	7.5	57.3	0.6

with gastric cancer and 16.8 ± 4.0 ng/ml for healthy volunteers and show no significant difference (P = 0.221; Fig. 2). The pharmacokinetic parameters of the plasma concentrations of tegafur, 5-FU, uracil and GBL after administration of UFT in the respective patients with gastric cancer are shown in Table 1, and the mean plasma concentration-time profiles of tegafur, 5-FU, uracil and GBL are shown in Figure 2. The values of Cmax for tegafur, uracil, 5-FU and GBL were 14.7 ± 5.2 and 4.0 ± 2.7 μg/ml, 191.2 ± 115.3 and 147.5 ± 57.3 ng/ml, respectively, and the values of Tmax were 1.0 ± 0.6, 1.1 ± 0.6, 0.9 ± 0.6 and 1.2 ± 0.6 hr, respectively (Table 1). The 5- FU concentration showed a similar time-profile of uracil, and the GBL concentration profile was similar to that of the parent drug, tegafur.

Discussion

The postoperative administration of UFT has been reported to prolong survival[6)~10)]. However, since the antitumor effect of UFT alone is not high, namely ranging from 9% to 20%[5)], it is considered that the inhibitory effects of UFT on angiogenesis are likely to be due to some other possible mechanism of action involved in the antitumor effect. Yonekura et al.[11)] and Basaki et al.[12)] reported that the antiangiogenic effects of UFT could be attributable to the actions of 5-FU, GHB and GBL, which are in chemical equilibrium under physiological conditions, and the latter exists in vivo predominantly as GHB (Fig. 1) while the inhibitory effects of GHB and GBL on angiogenesis are specific for VEGF[12)]. GHB suppresses the chemotactic migration and tube formation of human umbilical vein endothelial cells (HUVECs) stimulated by VEGF without inhibiting DNA synthesis in vitro. The way that GHB suppresses the VEGF related signal transduction pathway has been previously demonstrated. Moreover, the pharmacokinetics of the GBL concentration after the administration of UFT to patients still remain to be elucidated. The GBL concentration after the intravenous administration of tegafur, a component of UFT, has so far only reported by Au and Sadee[13)], but at 2000 mg/m²/day for 2 days by intravenous infusion over 30 min, the dose of tegafur was larger than the usual dose of UFT, and the concentration was determined at only one point about 2 hr after administration to only 2 patients. The present study clarified the baseline, as an endogenous GBL concentration in 10 gastric cancer patients and healthy volunteers, and the changes in the GBL concentrations after the administration of 200 mg of UFT twice a day orally on day 5 after the start to cancer patients for the first time. The Cmax obtained for GBL was 147.5 ng/ml(equivalent to 1.71μM), which was 5.5 times higher than the 50% inhibitory concentration (IC50, 0.31μM) of tube formation of human umbilical vein endothelial cells (HUVEC) with GBL reported by Basaki et al.[12)], and the concentration of 78.0 ng/ml (equivalent to 0.91μM), which was 3.0 times higher than the IC50, was maintained at 4 hr after administration.

Based on the pharmacokinetics of GBL, which were clarified in the present study, the concentration of GBL was much higher than its IC50 value for angiogenesis. Therefore, GBL is thus suggested to contribute to the anticancer effects of UFT in addition to that made by 5-FU, which is continuously metabolized from UFT. In combination with the continuous administration of 5-FU, which acts to inhibit angiogenesis[11)], it is thought that GBL has no

inhibitory effects on cell proliferation itself[11)12)].

GBL and GHB are endogenous compounds found in certain regions of both animal brains and the human brain where GHB may play a physiological role in the regulation of nerve activity, related with GABA[17)]. No differences in the endogenous concentration of GBL were observed between healthy volunteers and cancer patients. As a result, the change in GBL observed in this study seems to be due to the administration of oral UFT.

In addition to proliferating cancer cells and various types of normal cells, conventional cytotoxic chemotherapeutics affect the endothelium of the growing tumor vasculature. Kerbel et al.[18)] reported that the anti-angiogenic effects of chemotherapy seem to be optimized by the administration of such drugs 'metronomically' in small doses on a frequent schedule (daily, several times a week, or weekly) in an uninterrupted manner, for prolonged periods. Metronomic chemotherapy may significantly increase the anti-tumor effect when administered in combination with anti-angiogenic drugs, such as antibodies against VEGF or VEGF receptor-2[19)]. The oral administration UFT itself is a 'metronomic chemotherapeutic', because GHB and GBL themselves have anti-angiogenic potential. This might explain why UFT is more effective during the postoperative adjuvant phase[6)~10)], than in the treatment of metastatic disease[5)].

In conclusion, according to the pharmacokinetics of GBL, the metabolite of UFT may play an important role in the anti-angiogenetic action in gastric cancer patients. Future studies must examine the relationship between the blood GBL concentrations and the effects of the postoperative administration of UFT, as well as the relationship between the concentrations of VEGF in blood or tumors and the effects of UFT.

References

1) Fujii S, Kitano S, Fukushima M, Nakamura H, Maehara Y and Shirasaka T : Effect of Coadministration of Thymine of Thymidine on The Antitumor Activity of 1-(2-Tetrahydrofuryl)-5-Fluorouracil and 5-Fluorouracil. Gann 71 : 100-106, 1980.
2) Fujii S, Ikenaka K, Fukushima M and Shirasaka T : Effect of Uracil and Its Derivatives on Tumor Activity of 5-Fluorouracil and 1-(2-tetrahydrofuryl)-5-Fluorouracil. Gann 69 : 763-772, 1978.
3) Fujii S, Kitano S, Ikenaka K and Shirasaka T : Effect of Coadministaration of Uracil or Cytosine on the Tumor Activity of Clinical Dose of 1-(2-Tetrahydrofuryl)-5-Fluorouracil and Level of 5-Fluorouracil in Rodents. Gann 70 : 209-214, 1979.
4) Fujii S, Kitano S, Ikenaka K and Shirasaka T : Studies on Coadministration of Uracil or Cytosine on Antitumor Activity of FT-207 or 5-FU Derivatives(in Japanese). Gan to Kagakuryoho (Jpn J Cancer Chemother) 6 : 377-384, 1979.
5) Ota K, Taguchi T and Kimura K : Report on nationwide pooled data and cohort investigation in UFT phase II study. Cancer Chemother Pharmacol 22 : 333-338, 1988.
6) Kinoshita T, Nakajima T and Ohashi Y : National Surgical Adjuvant Study Group for Gastric Cancer (N-SAS-GC). Adjuvant Chemotherapy with Uracil-Tegafur (UFT) for Serosa Negative Advanced Gastric Cancer : Results of a Randomized Trial by National Surgical Adjuvant Study of Gastric Cancer. 41st ASCO Annual Meeting Abstract No.4021, 2005.
7) Lembersky BC, Wieand SH, Petrelli NJ, O'Connell MJ, Colangelo LH, Smith RE, Seay TE, Giguere JK, Marshall MA, Jacob AD, Colman LK, Soran A, Yothers G and Wolmark N : Oral Uracil and Tegafur Plus Leucovorin Compared With Intravenous Fluorouracil and Leucovorin

in Stage II and III Carcinoma of the Colon : Results From National Surgical Adjuvant Breast and Bowel Project Protocol C-06. J Clin Oncol 24 : 2059-2064, 2006.
8) Akasu T, Moriya Y, Ohashi Y, Yoshida S, Shirao K and Kodaira S : Adjuvant Chemotherapy with Uracil-Tegagur for Pathological Stage III Rectal Cancer after Mesorectal Excision with Selective Lateral Pelvic Lymphadenectomy : A Multicenter Randomized Controlled Trial. Jpn J Clin Oncol 36 : 237-244, 2006.
9) Kato H, Ichinose Y, Ohta M, Hata E, Tsubota N, Tada H, Watanabe Y, Wada H, Tsuboi M, Hamajima N and Ohta M : A Randomized Trial of Adjuvant Chemotherapy with Uracil-Tegafur for Adenocarcinoma of the Lung. N Engl J Med 350 : 1713-1721, 2004.
10) Noguchi S, Koyama H, Uchino J, Abe R, Miura S, Sugimachi K, Akazawa K and Abe K : Postoperative Therapy With Tamoxifen, Tegafur Plus Uracil, or Both in Women With Node-Negative Breast Cancer : A Pooled Analysis of Six Randomized Controlled Trials. J Clin Oncol 23 : 2172-2184, 2005.
11) Yonekura K, Basaki Y, Chikahisa L, Okabe S, Hashimoto A, Miyadera K, Wierzba and Yamada Y : UFT and Its Metabolites Inhibit the Angiogenesis Induced by Murine Renal Cell Carcinoma, as Determined by a Dorsal Air Sac Assay in Mice. Clin Cancer Res 5 : 2185-2191, 1999.
12) Basaki Y, Chikahisa L, Aoyagi K, Miyadera K, Yonekura K, Hashimoto A, Okabe S, Wierzba W and Yamada Y : Gamma-Hydroxybutyric acid and 5-fluorouracil, metabolites of UFT, inhibit the angiogenesis induced by vascular endothelial growth factor. Angiogenesis 4 : 163-173, 2001.
13) Au JL and Sadee W : Activation of Ftorafue [R,S-1-(tetrahydro-2-furanyl)-5-fluorouracil] to 5-fluorouracil and gamma-butyrolactone. Cancer Res 40 : 2814-2819, 1990.
14) Matsusima E, Yoshida K, Kitamura R and Yoshida K : Determination of S-1(combined drug of tegafur, 5-Chloro-2,4-dihydroxypyridine and potassium oxonate) and 5-fluorouracil in human plasma and urine using high-performance liquid chromatography and gas chromatography-negative ion chemical ionization mass spectrometry. J Chromatogr B 691 : 95-104, 1997.
15) Marunaka T, Umeno Y, Yoshida K, Nagamachi M, Minami Y and Fujuu S : High-Pressure Liquid Chromatographic Determination of Ftorafur [1-(Tetrahydro-2-furanyl)-5-fluorouracil] and GLC-Mass Spectrometric Determination of 5-Fluorouracil and Uracil in Biological Materials after Oral Administration of Uracil Plus Ftorafur. J Pharm Sci 69 : 1296-1300, 1980.
16) Fukui Y, Matsusima E, Muramoto K, Nagai N, Ohama K and Yamashita K : Validation of a simple gas chromatographic-mass spectrometric method for the determination of gamma-butyrolactone in human plasma. J Chromatogr B 785 : 73-80, 2003.
17) Doherty JD, Hattox SE, Snead OC and Roth RH : Identification of endogenous γ-hydrobutylate in human and bovine brain and its regional distribution in human, guinea pig and rhesus monkey brain. J Pharmacol Exp Ther 207 : 130-139, 1978.
18) Kerbel RS and Kamen BA : The antiangiogenic basis of metronomic chemotherapy. Nature Reviews 4 : 423-436, 2004.
19) Klement G, Baruchel S, Rak J, Man S, Clark K, Hicklin DJ, Bohlen P and Kerbel R S : Cotinuous low-dose therapy with viblastine and VEGF rexeptor-2 antibody induces sustained tumor regression without overt toxicity. J Clin Invest 105 : R15-R24, 2000.

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(和文抄録)

胃癌患者における経口フッ化ピリミジン UFT の体内動態の検討：血管新生阻害作用を示す代謝物 GHB と GBL を中心に

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経口フッ化ピリミジン系抗がん剤の一つである UFT（tegafur-uracil）は，近年，その代謝物である γ-hydrobutylic acid（GHB）と γ-butyronolactone（GBL）が，in vitro，in vivo で血管新生阻害作用を示すことが報告された．しかしながら，UFT 常用量における，これらの代謝物の体内動態は明らかにされていない．今回，我々は胃癌患者における UFT 内服後の GBL の動態を検討した．組織学的に確認され根治切除手術がなされた胃癌症例 10 例に対し，術後補助化学療法として UFT 400mg 2x daily が投与された．UFT 服薬開始後 5 日目，朝の服薬開始時点から 0，0.5，1，2，4 時間後に採血を施行した．対照として健常人ボランティア 10 名から，UFT の内服が無い状態で採血をおこなった(内因性 GBL 濃度)．Tegafur, 5-FU, uracil, GBL をガスクロマトグラフィー法にて測定した．内因性およびベースライン定常状態 GBL 濃度は，健常者で 16.8±4.0ng/ml，胃癌患者で 20.2±7.5ng/ml と差は無かった（p＝0.221）．Tegafur，5-FU，uracil，GBL の Cmax は 14.7 ± 5.2 and 4.0 ± 2.7 μg/ml，191.2 ± 115.3 and 147.5 ± 57.3 ng/ml であり，Tmax は 1.0 ± 0.6，1.1 ± 0.6，0.9 ± 0.6 and 1.2 ± 0.6 hr であった．GBL のヒト血漿中体内動態は，それが in vitro において，血管新生抑制作用を示す IC50＝25.8ng/ml よりも十分に高い濃度に維持されることが証明された．UFT の示す活性に，主代謝物である 5-FU の抗腫瘍活性，血管新生抑制作用に加えて，直接の抗腫瘍活性を持たない副代謝物の GHB，GBL が血管新生抑制作用を介して関与している可能性が示唆された．