can predict the chemosensitivity of tumor cells within 5 days, this system has the same drawback as the clonogenic assay in soft agar.

Our cell mat system offers several unique properties: (a) the assay does not limit the periods of drug exposure, and therefore, single or combined modality treatment is feasible; (b) the cell number used is less than one-fifth that needed for the soft agar assay, which is important because a limited amount of tissue is available (especially from tumors detected in early stages); (c) objective data can be obtained within 5 days. However, it is not known whether the cell mat assay selectively measures the chemosensitivity of self-renewing stem cells within tumors, which may relate to the patient's response to chemotherapy. Collaborative studies comparing clinical response to drugs with the chemosensitivity obtained by the cell mat assay are under way.

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Reversal of 5-Fluorouracil-Induced Myelosuppression by Prolonged Administration of High-Dose Uridine

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The effect of high-dose uridine on 5-fluorouracil (5-FU)-induced toxicity was investigated. Nine patients were treated

weekly with 5-FU at increasing dosages. Five patients developed dose-limiting leukopenia, and four patients developed thrombocytopenia. At dose-limiting toxicity, 5-FU treatment was repeated and followed after 3 hours by intermittent iv infusion of uridine (2 g/m² per hr) during 72 hours. Leukopenia was reversed for several weeks but thrombocytopenia was not. Side effects consisted of mild rises in body temperature. The pharmacokinetics of uridine were similar to those observed with single-agent uridine. Our data indicate that high-dose uridine can reduce the severity of 5-FU-induced myelosuppression. [J Natl Cancer Inst 1989;81:157-162]

Treatment with single-agent 5-fluorouracil (5-FU) is considered to be the standard therapy for advanced colorectal cancer. However, the response rate does not exceed 20%, and no significant influence on survival has been obtained. The mechanisms of action of 5-FU have been investigated extensively and are complex. The 5-FU metabolite 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP) inhibits the enzyme thymidylate synthase, resulting in a decreased DNA synthesis (1). A second mechanism of action of 5-FU involves the incorporation of 5-fluoro-uridine-5'-triphosphate (FUTP) into RNA (2), leading to impaired processing of nuclear RNA (3). Also, 5-fluoro-2'-deoxyuridine-5'-triphosphate (FdUTP) recently has been shown to be incorporated into DNA (4).

Data support the hypothesis that biochemical modulation of 5-FU metabolism by uridine may enhance the

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therapeutic index of 5-FU by prevention of its toxicity. Both Martin et al. (5) and Klubes et al. (6,7) demonstrated that tumor-bearing and non-tumor-bearing mice tolerated much higher doses of 5-FU when administration of the fluoropyrimidine is followed by high doses of uridine. In the latter studies, prolonged exposure of the mice to uridine appeared to be a prerequisite.

The precise biochemical mechanism by which uridine protects against 5-FU-induced toxicity is as yet unknown. From studies in CD8F₁ murine breast cancer, Sawyer et al. (8) suggested that protection from 5-FU-induced toxicity is mediated by competition of 5-FU and uridine metabolites for incorporation into RNA and not for the effects on DNA.

Studies that evaluate the potential of uridine to rescue normal tissues in humans have not been performed previously. We recently reported our initial experiences on the application of high-dose uridine in man (9). Uridine was administered as 1-hour iv infusions at a dose of 1–12 g/m². The only toxic effect observed consisted of transient shivering at the highest dose levels. Concurrent pharmacokinetic studies revealed that peak plasma concentrations of approximately 2 mM were obtained. However, elimination was rapid, resulting in short-term exposure of the tissues to high concentrations of the nucleoside. When this schedule of uridine was administered after a myelotoxic dose of 5-FU, no rescue of bone marrow suppression was observed in two patients. Therefore, studies were conducted with prolonged infusions of uridine (10). Continuous infusion of uridine with the aim to achieve plasma uridine concentrations of about 1 mM was not feasible due to a rapid rise in body temperature. In an attempt to overcome this side effect, but with maintenance of high plasma uridine concentrations, the nucleoside was administered intermittently at a dose of 1–3 g/m² per hr in a schedule in which a 3-hour infusion was alternated with 3-hour infusion-free periods over a 72-hour period. This schedule appeared to be feasible since fever was no longer dose limiting. The intermittent infusion produced plasma uridine concentrations that were in the

millimolar range. However, during the treatment-free intervals, plasma levels decreased to a 100–350 μ M range.

The clinical effects of uridine on 5-FU-induced bone marrow suppression are described.

Materials and Methods

Patients

The characteristics of nine patients with advanced cancer who participated in the study are depicted in table 1. All patients had advanced colorectal cancer except for one (No. 9) with breast cancer. They were in good general condition, with a median performance status [World Health Organization (WHO) standard] of 1, and had normal renal, hepatic, and bone marrow function. Informed consent was obtained in each case. Four of the patients had received prior chemotherapy.

Treatment Schedule

Initially the patients received weekly iv bolus injections of 5-FU on an outpatient basis. The starting dose was

500 mg/m². Every 4 weeks the 5-FU dose was escalated by 20% until dose-limiting toxicity occurred, which consisted of myelosuppression in all patients. At the time toxicity became evident, the patients were hospitalized and a central venous catheter was inserted because previous experience had shown that prolonged administration of uridine in peripheral veins caused phlebitis (10). Detailed information on the toxic dose levels of 5-FU and the degree of leukopenia or thrombocytopenia is shown in table 2.

During hospitalization, weekly 5-FU treatment was continued at the dose that produced myelosuppression. However, 5-FU was now followed after 3 hours by uridine at a dose of 2 g/m² per hr given as intermittent iv infusions over 72 hours and consisting of 3-hour infusions of uridine alternated with 3-hour uridine-free periods. During the uridine administration, body temperature was recorded every 3 hours.

Patients were discharged from the hospital on the day after the end of the uridine infusion and were seen

Table 1. Characteristics of patients given 5-FU and uridine

Patient No.	Age (yr)	Sex	Performance status (WHO)	Prior chemotherapy*
1	57	F	1	None
2	70	F	1	None
3	66	F	0	HAI 5-FU
4	62	M	2	None
5	61	F	1	None
6	55	M	1	HAI 5-FU
7	74	M	1	TGU
8	55	M	1	None
9	49	F	0	FAC

*HAI = hepatic arterial infusion, TGU = 1,2,4-triglycidylurazol, FAC = 5-fluorouracil-doxorubicin-cyclophosphamide.

Table 2. Toxic dose of weekly 5-FU and severity of myelosuppression

Patient No.	Toxic dose (mg/m ²)	Nadir at toxicity	
		wbc ($\times 10^6$ /mL)	Platelets ($\times 10^6$ /mL)
1	720	1.6	151
2	600	2.7	101
3	600	1.4	76
4	864	2.8	110
5	864	2.0	205
6	864	4.3	84
7	600	6.7	82
8	720	4.1	95
9	720	3.7	74

again weekly at the outpatient clinic. They were examined according to medical history, physical condition, complete blood cell count, serum creatinine, bilirubin, alkaline phosphatase, gamma-glutamyltransferase, aspartate aminotransferase, alanine aminotransferase, and lactic acid dehydrogenase. We attempted to continue the 5-FU treatment in the same weekly schedule while maintaining the dose level responsible for the above-mentioned bone marrow cytotoxicity. The patients were removed from the study as soon as dose reduction of 5-FU was required, whatever the reason.

Uridine Pharmacokinetics

Plasmas of patients on protocol were analyzed for uridine and uracil. Blood was drawn in heparinized tubes before the start of the uridine infusion and at various times corresponding with either the start or the end of a 3-hour uridine administration period. Plasma was separated by centrifugation and stored at -20°C . After deproteinization with trichloroacetic acid followed by neutralization, uridine and uracil levels were determined by means of reversed-phase high-performance liquid chromatography as described earlier (9,11).

Results

Effect of Uridine on 5-FU-Induced Leukopenia

After weekly single-agent bolus injections of 5-FU, patients No. 1-5 developed leukopenia. This occurred at dose levels of $600\text{--}864\text{ mg/m}^2$. The nadirs of the wbc's ranged from 1.4 to $2.8 \times 10^6/\text{mL}$ (table 2, fig. 1). Only one of these patients had received previous chemotherapy. At the time of this leukopenia, 5-FU treatment was continued while followed by uridine for 72 hours. As can clearly be seen from figure 1, the wbc's in these five patients increased markedly to maximum values ranging from 2.9 to $6.2 \times 10^6/\text{mL}$, despite continued 5-FU administration. Interestingly, this effect of uridine on 5-FU-induced leukopenia lasted for several weeks, although uridine was administered only after one dose of 5-FU. The time course of the platelets of patients 1-5 is shown in figure 2. At the time of uridine administration, all but one of these patients had a platelet count $>100 \times 10^6/\text{mL}$ (range, $76\text{--}205$). As with the wbc's, a rise in the platelet count was observed. However, the magnitude of this rise was lower than that of the wbc's, but the du-

Figure 1. Course of wbc count for the weeks immediately prior to and following uridine treatment in patients 1-5. Patients are indicated by different symbols. *Small open bars* represent weekly 5-FU treatment. *Large closed bar* represents intermittent uridine infusion during 72 hr. — is added to weeks prior to uridine infusion and + to weeks after infusion.

ration of this effect lasted for a shorter time.

Effect of Uridine on 5-FU-Induced Thrombocytopenia

Effect of Uridine on 5-FU-Induced Thrombocytopenia

The other four patients (No. 6-9) developed thrombocytopenia during

Figure 2. Course of platelets for the weeks immediately prior to and following uridine treatment in patients No. 1-5. Different symbols indicate the same patients as in fig. 1. *Small open bars* represent weekly 5-FU treatment. *Large closed bar* represents intermittent uridine infusion during 72 hr. — is added to weeks prior to uridine infusion and + to weeks after infusion.

weekly 5-FU treatment at dose levels of 600–864 mg/m². The platelet count varied from 74 to 95 × 10⁶/mL (table 2, fig. 3). Three of these four patients had been exposed to previous chemotherapy. Only the patient who had not received prior chemotherapy showed a minor increase in the platelet count from 84 to 108 × 10⁶/mL. In the remaining three patients of this group, uridine failed to reverse 5-FU-induced thrombocytopenia. A further decrease of the platelets required discontinuation of the 5-FU treatment after 1–3 weeks. After uridine administration, no change in the wbc count was observed in the thrombocytopenic patients (data not shown).

Toxicity of Combined 5-FU-Uridine Administration

All nine patients tolerated the treatment very well. There have been no signs or symptoms that indicated that the addition of uridine added any subjective side effect to the weekly 5-FU treatment. The only toxicity that was observed consisted of minor-to-moderate rises in body temperature that did not require therapy or discontinuation of uridine. The median rise in body temperature of the nine patients during the 72-hour uridine administration was 1.0 °C (range, 0.4–1.7). No other side effects were observed.

Pharmacokinetics of Uridine

Plasma concentrations of uridine and uracil were determined in eight of the nine patients (No. 1–4 and No. 6–9). In pretreatment plasma, uridine concentration was 1.5–9 μM. Peak uridine concentrations (at the end of the 3-hr infusion) ranged from 521 to 987 μM, while nadir concentrations (at the end of the 3-hr uridine-free period) were 138–670 μM. Corresponding values for the plasma uracil concentrations were 77–811 μM and 47–532 μM, respectively (table 3). These values were comparable to those determined in the phase I study of single-agent uridine (10).

Discussion

A potential and attractive method to improve the poor therapeutic results of metastasized colorectal cancer may result from the application of metabolic modulation to enhance the therapeutic index of 5-FU (12,13). A number of naturally existing substances and cytotoxic drugs may be useful in this respect.

The present study is the first to describe the potential value of uridine in the metabolic modulation of 5-FU activity in cancer patients with advanced disease. Our clinical findings agree with the preclinical data of Martin et al. (5) and those of Klubes et al. (6,7), who

reported that mice can be “rescued” from lethal toxicity of 5-FU by delayed administration of uridine. Recently, we reported similar results on the use of uridine rescue in mice bearing murine colon 26 carcinoma (14). In the latter experiments, the maximum tolerable dose of 5-FU could be increased 2.5- to 3-fold when 5-FU was combined with high-dose uridine. This resulted in a superior antitumor effect and an increase in life span when compared to the effects of single-agent 5-FU.

Our data show reversal of 5-FU-induced leukopenia in five of five patients. Under ordinary clinical circumstances, 5-FU treatment would have been postponed for one or more weeks until recovery of wbc count. Co-administration of uridine with weekly doses of 5-FU, given at the “toxic” dosage, resulted in an increase in cumulative dose of 5-FU over a period of 3–5 weeks. Whether this will be translated into a more effective treatment is not yet known. Of interest, the effect of one cycle of uridine lasted several weeks. On the basis of the theory that RNA-FUTP needs to be replaced by uridine-triphosphate (UTP) to reverse 5-FU-induced toxicity, we hypothesize that UTP tissue pools remain oversaturated for a period of several weeks.

The failure of uridine to reverse 5-FU-induced thrombocytopenia has yet to be explained, but this may be based on a mere difference in uridine metabolism in neutrophil precursors and megakaryocytes in humans. However, in mice we also observed prevention of 5-FU-induced thrombocytopenia by uridine (14). Another factor that may have affected the results in the present study is the fact that three of four patients who developed thrombocytopenia had received prior chemotherapy, while only one of five leukopenic patients was pretreated. Whether prior chemotherapy does play a role needs to be elucidated in future studies that include larger numbers of patients.

An important potential aspect of the application of uridine in the prevention of toxicity of 5-FU is selectivity. Indeed, in the experiments in tumor-bearing mice mentioned earlier (5,7,14), uridine protected against 5-FU toxicity while the antitumor activity of 5-FU was en-

Figure 3. Course of platelets for the weeks immediately prior to and following uridine treatment in patients No. 6–9. Patients are indicated by different symbols. Small open bars represent weekly 5-FU treatment. Large closed bar represents intermittent uridine infusion during 72 hr. — is added to weeks prior to uridine infusion and + to weeks after infusion.

Table 3. Plasma uridine and uracil concentrations during intermittent uridine infusions given at the toxic dose of 5-FU

Patient No.	Uridine concentration (μM)*		Uracil concentration (μM)*	
	Peak	Nadir	Peak	Nadir
1	797 $\pm$ 46	191 $\pm$ 21	574 $\pm$ 59	440 $\pm$ 20
2	847 $\pm$ 151	670 $\pm$ 39	485 $\pm$ 56	532 $\pm$ 3
3	987 $\pm$ 111	n.d.	811 $\pm$ 43	n.d.
4	600 $\pm$ 37	247 $\pm$ 19	77 $\pm$ 22	47 $\pm$ 13
6	521 $\pm$ 29	163 $\pm$ 26	226 $\pm$ 17	181 $\pm$ 33
7	599 $\pm$ 60	241 $\pm$ 36	319 $\pm$ 102	326 $\pm$ 74
8	802 $\pm$ 91	469 $\pm$ 57	489 $\pm$ 35	432 $\pm$ 72
9	846 $\pm$ 36	138 $\pm$ 23	228 $\pm$ 36	161 $\pm$ 26

*Values = mean $\pm$ SD; n.d. = not determined.

hanced, resulting in an improvement of the therapeutic index of 5-FU. The selectivity of the effect of uridine might be related to the effect of 5-FU on RNA of normal cells (8), but other factors may also be involved. Firstly, it has been demonstrated that at physiological uridine levels, uridine conversion to nucleotides proceeds at a higher rate in blood cells than in other cells (15). Secondly, during uridine administration bone marrow cells are probably better exposed to the high uridine concentrations. Because uridine kinase activity is relatively high in these cells (8,16), uridine is converted to nucleotides at an increased rate, resulting in a higher ratio of total uridine nucleotides versus total fluorouridine nucleotides in bone marrow cells than in cells of solid tissues. Thus, selectivity of uridine rescue may be explained by a number of potential differences between normal cells and tumor cells.

The side effects of uridine observed in the present study were similar to those we observed with single-agent uridine (10) and consisted mainly of a mild rise in body temperature. Interestingly, in a study in rabbits, ip or iv administration of uridine and its catabolites also caused significant rises of body temperature, suggesting a causal relationship (17). However, the exact role of uridine or its catabolites in the observed temperature changes remains to be defined.

The use of the uridine phosphorylase inhibitor benzylacyclouridine with low-dose uridine might be an alternative for future clinical studies since this appears to diminish uridine-induced temperature effects (18) and efficiently enhance pools of uridine and its nucleotides in

tissues (19). When the pharmacokinetic data of the present study were compared with those obtained with single-agent uridine (10), the results did not appear to be affected by the concomitant administration of 5-FU.

Since iv administration of high-dose uridine requires admittance of the patient to the hospital, we have initiated a similar study with oral uridine. However, studies by Klubes et al. (20) in mice and also our initial findings in humans (21) disclosed that plasma uridine concentrations are much lower following oral administration than those observed after parenteral administration of the nucleoside. Bioavailability with the oral route appears to be $<10\%$ in both species. Further studies will reveal whether these lower uridine concentrations are capable of protecting against 5-FU-induced toxicity.

In conclusion, it has yet to be demonstrated whether the biologic effect of uridine will prove to be selective in humans as well as in mice and whether the nucleoside will result in a tool to improve the therapeutic index of 5-FU. Our findings warrant clinical studies in which 5-FU is combined with uridine from the start of treatment and finally through trials that compare the therapeutic efficacy of the combination with that of single-agent 5-FU.

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Allium Vegetables and Reduced Risk of Stomach Cancer

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Interviews with 564 patients with stomach cancer and 1,131 controls in an area of China where gastric cancer rates are high revealed a significant reduction in gastric cancer risk with increasing consumption of allium vegetables. Persons in the highest quartile of intake experienced only 40% of the risk of those in the lowest. Protective effects were seen for garlic, onions, and other allium foods. Although additional research is needed before etiologic inferences can be made, the findings are consistent with recent reports of tumor inhibition following administration of allium compounds in experimental animals. [J Natl Cancer Inst 1989;81:162-164]

Allium vegetables, including garlic and onions, have been used for 3,000 years as flavor-enhancing foods and folk medicines. Their biological effects against bacteria and fungi in treatment of various diseases, especially of the digestive tract, have been demonstrated (1). Recently, attention has been paid to the pharmacologic activity of allium extracts and oils in the inhibition of carcinogenesis. Animal and in vitro experiments indicate that compounds in allium vegetables (e.g., allyl sulfides) inhibit several types of tumors and decrease tumor growth and proliferation (2-9). Little information is available,

however, on their effects in humans. Herein, we present data on the effects of dietary intake of allium vegetables from a large population-based case-control study performed in a population at high risk for stomach cancer in Shandong Province, People's Republic of China.

Patients and Methods

The study design has been detailed elsewhere (10,11). In brief, between 1984 and 1986, interviews were sought with all newly diagnosed stomach cancer patients aged 35-69 years and with controls of similar sex and age in Linq County, Shandong Province, an area with one of the highest stomach cancer mortality rates in China; annual age-adjusted rates per 100,000 were 55 for males and 19 for females in 1980-1982. The patients were Linq residents identified from a specially established reporting system involving county and commune hospitals whose coverage of stomach cancer incidence was thought to be essentially complete. The controls were randomly selected from age and sex strata of the Linq population with the use of census rosters available in each village. Twice as many controls as cases were selected and were chosen to have a similar age and sex distribution to that of the cases.

A structured questionnaire was developed for use in this study after pilot testing in Linq. The questionnaire sought information on the frequency of intake of, and portion size for, 85 food items consumed several years prior to interview (1980) and just prior to the Cultural Revolution (1965). Specific questions were asked concerning intake of five vegetables of the allium class: garlic, garlic stalks, scallions, Chinese chives, and onions. Individuals were then grouped into approximately equal categories (usually quartiles or tertiles) based on their yearly consumption of individual or total allium vegetables. The measure of association between stomach cancer risk and allium intake was the odds ratio (OR). Summary ORs were estimated (a) by the Mantel-Haenszel technique adjusting for sex, age, and family economic situation and (b) by logistic regression analyses (12) adjusting for these and several potential confound-

ing factors, including consumption of non-allium vegetables, previously identified in this study (11).

Results

A total of 685 patients with stomach cancer were identified over the study period. Interviews were completed with 564 (82%) patients; excluded were 41 who had died, 70 who were too ill, eight who refused an interview, and two who had incomplete interviews. Fifty percent of the cancer diagnoses were based on histologic review of tissue specimens obtained from surgery or endoscopy, 32% on surgery or endoscopy without tissue review, and the remaining 17% on radiologic or clinical grounds. Among those diagnoses with pathologic confirmation, the ratio of intestinal-to-diffuse-type cancers was 4.7:1. Most (63%) of the cancers were found in the antrum of the stomach.

Interviews were completed with 1,131 controls, with only one person contacted refusing to participate. The distributions of patients and controls were similar with respect to sex, age, place of birth (96% were born in Linq), occupation, and education. The patients, however, had significantly lower family incomes; thus, the analyses were adjusted for income.

Table 1 shows that consumption of allium vegetables in 1980 was less

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