known morphology, morphometry, rigidity, surface-marker phenotype, cytoxic activity, growth characteristics, and adhesive properties (4-6,20). The use of cell populations native to the rabbit would require extensive characterization and may be of little value, since these cells may not share many characteristics with relevant human cell populations. Since the injection of A-LAK cells with IL-2 did not result in any noticeable change in the normal ear chamber, it seems unlikely that the xenogenic system contributed to the phenomena observed in this study.

In conclusion, this study has shown that A-LAK cells may possess some degree of selectivity for prolonged attachment to tumor microcirculation. Since one endothelial cell supplies nutrients to several thousand tumor cells, targeting these therapies to endothelial cells may provide a novel antiangiogenic approach to cancer treatment (17). This mechanism may indeed be responsible for tumor regression seen in patients (1) despite delivery of small numbers of cytotoxic cells to tumors. On the other hand, if the blood vessels are collapsed or arteriovenous shunts are present (both occur in tumors during growth) (19), these cells may not arrive in poorly perfused regions of the tumors. These peculiarities of tumor vasculature may also explain why some tumors do not respond to adoptive immunotherapy. Therefore, the dynamics of effector cell interaction with the tumor vasculature must be taken into account in the development of improved strategies for adoptive immunotherapy.

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Clinical and Pharmacologic Study of Orally Administered Urudine

C. J. van Groeningen,*, G. J. Peters, J. C. Nadal, E. Laurense, H. M. Pinedo

Effects of oral administrations of uridine were investigated in a study of six healthy volunteer control subjects and nine patients with metastatic colorectal cancer. Oral uridine was studied as single-dose administrations at doses escalating from 0.3 to 12 g/m² and as multiple-dose administrations every 6 hours for 3 days at doses from 5 to 10 g/m². The maximum tolerated dose (MTD) was 10 to 12 g/m² for a single dose of uridine and 5 g/m² for the multiple-dose regimen. Diarrhea was the dose-limiting toxic effect. Single-dose oral uridine resulted in an increase in plasma uridine concentrations in the range of 60 to 80 μM after doses of 8 to 12 g/m². At these doses, bioavailability of oral uridine ranged from 5.8% to 9.9%. At the MTD of 5 g/m² in the multiple-dose uridine schedule, steady-state plasma uridine levels of approximately 50 μM were achieved. Further studies should explore the role of oral uridine in the

Fluorouracil (5-FU) is used for the treatment of various malignant tumors. Its antitumor activity, however, is of limited value. Biochemical modulation (1-3) offers a potential method for enhancing the therapeutic index of antineoplastic agents. It has been shown in tumor-bearing mice that delayed administration of high-dose uridine improves the therapeutic index of 5-FU as well as that of 5-FU combined with leucovorin calcium (4-8).

On the basis of the murine studies, we performed a number of clinical investigations with the goal of enhancing the therapeutic index of 5-FU in humans (9-11). Initially, uridine was evaluated in a phase I study as 1-hour infusions at doses in the range of 1 to 12 g/m² (9). The dose-limiting toxic effect was transient shivering. Millimolar peak plasma concentrations of uridine were obtained, but elimination was very rapid. Because a more prolonged exposure of the tissues to high concentrations of uridine was believed necessary to achieve the desired effect, we subsequently studied prolonged infusions of uridine (10). Continuous infusion of uridine appeared to be impossible because of the rapid onset of high fever. When uridine was administered as intermittent infusions, however, fever was no longer dose limiting.

Intermittent uridine infusion produced plasma uridine concentrations in the millimolar range. During the treatment-free intervals, however, plasma levels decreased to a range of 100 to 350 μM. Encouraging results were obtained in a later study in which patients with advanced colorectal cancer were treated with weekly bolus injections of 5-FU (11). In this study, it was shown that 5-FU-induced leukenia, but not thrombocytopenia, could be reversed by administrations of uridine.

A major drawback of high-dose parenteral uridine administration is that patients must be admitted to the hospital. Moreover, when uridine is administered by peripheral vein infusion, severe phlebitis of the infused vein will rapidly occur (10). Because of the drawbacks associated with these procedures, it would be attractive to study the potential of oral administration of uridine. Klues et al. (12) reported the results of oral uridine administration in mice, for which the bioavailability of oral uridine was low (7%) compared with that resulting from parenteral administration of uridine. However, prolonged and relatively constant uridine levels were obtained. Martin et al. (13) found that high-dose oral uridine effecuted abatement ("rescue") of 5-FU-induced toxic effects comparable to that obtained with the use of parenteral uridine. When the uridine phosphorylase inhibitor 5-benzylcyclopropuridine was added to the combination of 5-FU and uridine, a dose reduction of 50% of the uridine achieved the same rescue effect. This report discusses the clinical and pharmacokinetic aspects of administration of oral uridine to humans.

Subjects and Methods

Subjects. The characteristics of six healthy volunteer control subjects and nine patients with advanced colorectal cancer are depicted in Table 1. Each of the nine patients was in good general condition, with a World Health Organization (WHO) median performance status of 1 and normal renal function. All nine patients had liver metastases and elevated liver enzymes but normal levels of serum bilirubin. Informed consent was obtained from each subject.

Uridine administration. The six healthy control subjects and three of the patients with colorectal cancer received one or more single administrations of oral uridine (prepared as a 20% solution in water by the hospital pharmacy) at doses ranging from 0.3 to 12.0 g/m² (Table 1). To avoid the effect of a possible circadian variation in uridine catabolism, we administered uridine between 9 and 11 AM. The other six patients with colorectal cancer received repeated administrations of oral uridine (Table 1). In this schedule, uridine was given every 6 hours for a total of 12 administrations. Two patients received a dose of 5 g/m² each; one patient received a dose of 8 g/m²; the other three patients were started at doses of 8 and 10 g/m² that were subsequently

<table>
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<tr>
<th>Subject No.</th>
<th>Category of subject*</th>
<th>Age, y</th>
<th>Sex</th>
<th>Uridine dose, g/m²</th>
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<td></td>
<td></td>
<td></td>
<td>Single dose</td>
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<td>P</td>
<td>33</td>
<td>Male</td>
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</tr>
</tbody>
</table>

*HV = healthy volunteer control subject; P = patient. † >> = decreased to.

Received July 20, 1990; revised December 12, 1990; accepted December 27, 1990.
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decreased to 5 g/m² after the appearance of toxic effects.

**Uridine pharmacokinetics.** Plasma samples from the healthy control subjects and from the patients were analyzed for the presence of uridine and uracil by high-pressure liquid chromatography. From those receiving single-dose administration of uridine, blood was drawn before the oral administration of uridine and at 0.5, 1, 2, 2.5, 3, 4, 6, 8, and 10 hours thereafter. For those receiving multiple-dose administrations of uridine, blood was drawn before and at various times during the 3 days of uridine administration. Plasma was separated from heparinized blood by centrifugation and stored at -20°C. Levels of uridine and uracil in plasma were determined as previously described (14).

**Results**

**Toxic Effects of Single-Dose Oral Uridine**

At all dose levels, the uridine administered orally was well tolerated. Those subjects who received uridine at doses from 0.3 to 8 g/m² did not experience side effects. However, at a dose of 10 g/m², one subject experienced abdominal cramps, while two others (one healthy control subject and one patient) had diarrhea (WHO grade 1). Also, at the uridine dose of 12 g/m², one healthy control subject experienced abdominal cramps, and both other subjects (healthy control subjects) experienced grade 1 diarrhea. No other side effects were observed.

**Toxic Effects of Repeated-Dose Oral Uridine**

In this schedule, the first patient was started on uridine at a dose of 10 g/m². Three episodes of diarrhea were observed beginning at 2 hours after the oral intake. Therefore, each subsequent uridine administration was given at a dose of 5 g/m²; diarrhea did not recur. The second patient received uridine at a dose of 8 g/m². Only very mild diarrhea occurred, mainly on the first day, and this effect did not require a reduction in the dose. The third patient received uridine at a dose of 8 g/m², but because of the onset of diarrhea, the dose was reduced to 5 g/m² after six administrations; the diarrhea then subsided. The fourth and fifth patients on the multiple-dose uridine schedule received 5 g/m² each. Both patients experienced some loosening of their stools but no real diarrhea. The sixth patient was initially given uridine orally at a dose of 8 g/m². After five administrations, however, diarrhea was observed, and the dose was decreased to 5 g/m². At this dose, no diarrhea occurred.

Except for diarrhea, other side effects, including fever, were not observed when uridine was given in this multiple-dose regimen.

**Pharmacokinetics of Oral Uridine**

**Single-dose uridine.** The uridine concentration in plasma before treatment varied between 2.8 and 7.8 μM, whereas the concentration of uracil was less than 1 μM. In Fig 1, representative curves for concentration versus time for the single-dose uridine administration are shown for all dose levels. Peak uridine plasma concentrations for the higher dose levels (8 to 12 g/m²) were 60 to 80 μM, values that were reached after 2 to 5 hours. Apparently, the absorption of uridine from the gastrointestinal tract was saturated at dose levels of 8 g/m², since higher doses did not result in increased peak levels. Absorption and elimination appeared to be in equilibrium.

Plasma uracil concentrations in the patients were not detectable nor were they comparable to those in the control subjects for uridine doses less than 8 g/m². At a uridine dose of 8 g/m², uracil concentrations increased to approximately 12 μM after 5 hours. At the dose level of 10 g/m², a large variation was observed. In one subject (No. 9), plasma uracil levels increased to 2 μM; in two subjects (No. 4 and 5), levels increased to 10 and 15 μM, respectively, after 9 hours. At the maximum tolerated dose (MTD) of 12 g/m², the plasma uracil levels increased to 86 μM in one subject (No. 7), while in the other two subjects (No. 3 and 8), only small increases in plasma uracil levels were observed (Fig 2). A linear relationship was observed between uridine dose and area under the concentration-versus-time curve. This rela-

![Fig 1. Curves for plasma concentration of uridine versus time after administration of single-dose oral uridine at 0.3-12 g/m². Various doses are indicated by different symbols. For the 12-g/m² dose, the curve for subject 8 is shown.](image-url)
tionship had a correlation coefficient of .71 for all values and all subjects and .98 for the only subject (No. 3) who received uridine at four different dose levels.

Table 2 summarizes the pharmacokinetic parameters of single-dose uridine. The mean residence time was 244 ± 39 minutes and appeared to be independent of the dose of uridine. Plasma clearance had a mean value of 41 ± 19 mL/kg per minute, also unrelated to dose. The volume of distribution was 11.5 ± 4.5 L/kg. The bioavailability was low when compared with that of a single intravenous (IV) dose of uridine (9), ranging from 5.8% to 9.9% for dose levels of 8 to 12 g/m². Also, for these dose levels, the level of uridine excreted in the urine was low (±1% of the dose). The levels of uracil in the urine could not be determined accurately because of interfering peaks in the chromatogram.

Multiple-dose oral uridine. During repeated administrations of oral uridine at the doses used (5, 8, and 10 g/m²), plasma uridine concentrations increased to steady-state levels of 30 to 75 µM. These levels were reached after approximately 4 hours. Plasma uracil concentrations among the patients showed large variations, ranging from 10 to 200 µM. Uracil formation was apparent only after approximately 6 hours (Fig 3). At the determined MTD of 5 g/m², steady-state plasma uridine concentrations were on the order of 50 µM. Representative curves for concentration versus time for patients No. 13 and 14 are depicted in Fig 3.

Discussion

Biochemical modulation of 5-FU by means of the delayed administration of high-dose uridine is performed on the basis of the hypothesis that 5-FU-induced toxic effects may be correlated with the incorporation of 5-fluorouridine 5'-triphosphate into RNA. The mechanism of the rescue of 5-FU-induced toxic effects is not obvious. When uridine is given several hours after administration of 5-FU, uridine triphosphate may replace 5-fluorouridine 5'-triphosphate in RNA.

It has also not been established what plasma levels of uridine are necessary to reverse the toxicity of 5-FU. Parenteral uridine administration to mice produced plasma concentrations of uridine of approximately 10 mM (4,15), whereas in patients and rabbits, peak levels were approximately 1 to 2 mM (10,14). It was clear from the data from experiments with mice done by Martin et al (13), however, that such extremely elevated levels are not necessary, a finding possibly related to the mechanism of uridine access. Uridine can be taken up by cells through a facilitated diffusion mechanism (16) and through a concentration Na⁺-dependent mechanism as reported by Darnowski and Handschumacher (17). It has been demonstrated that uridine concentrations in several tissues are significantly higher than those in plasma (15,17,18). It has also been demonstrated that uridine concentrations as used in vitro, which are similar to plasma uridine concentrations as observed in patients after administration of oral uridine, can increase nucleotide pools in L1210 cells by 50% to 100% (19). From these data, it was concluded that the maintenance of moderately elevated plasma concentrations rather than short-lasting, very high peak levels of uridine is critical to induce abatement of 5-FU-induced toxic effects.

The dose-limiting toxic effect in the present study was diarrhea. No fever was observed in the subjects receiving uridine.

![Fig 2. Curves for plasma concentration of uracil versus time for the three subjects who received oral uridine at a dose of 12 g/m². Various subjects are indicated by different symbols: ∆, subject 3; ◇, subject 7; x, subject 8.](image)

![Fig 3. Curves for plasma concentration of uridine versus time for the two patients (13 and 14) who received 5 g/m² of oral uridine in the multiple-dose regimen. Various patients are indicated by different symbols. Arrows indicate the time points of uridine administration.](image)
orally, in contrast to results of studies in which it was administered intravenously. This absence of fever might be explained by peak plasma concentrations of uridine and its catabolite uracil associated with oral administration that are lower than those associated with IV administration \((10,11,14)\). These levels are possibly too low to affect thermoregulation, which was shown to be dose and concentration dependent in humans, mice, rats, and rabbits \((10,14,15)\).

The uridine pharmacokinetics after administration of oral uridine in the subjects of this study were similar to the uridine pharmacokinetics in the mouse \((12)\). For those subjects on the single-dose schedule, peak plasma concentrations were much lower than those observed in patients undergoing 1-hour IV infusion. For the highest doses of IV \((12 \text{ g/m}^2)\) and oral \((12 \text{ g/m}^2)\) uridine, peak levels of 2000 \(\mu M\) and 80 \(\mu M\), respectively, were achieved. For oral uridine at doses of 8 to 12 \(\text{g/m}^2\), bioavailability ranged from 5.8% to 9.9%. A comparable bioavailability of 7% was observed in the mouse, as reported by Klubes et al \((12)\).

At the MTD of 5 \(\text{g/m}^2\) in the multiple-dose oral uridine regimen, constant plasma uridine levels of approximately 50 \(\mu M\) were maintained during a 3-day period. In the mouse, concentrations of this magnitude were inadequate for rescue purposes \((13)\). When, however, 5-benzylacyclouridine, an inhibitor of uridine phosphorylase, was added to uridine and uridine levels greater than 70 \(\mu M\) could be maintained during a prolonged period, 5-FU-induced toxic effects were prevented effectively. As stated above, it is not yet known which plasma uridine levels are required for modulation of 5-FU-induced toxic effects in humans. Nevertheless, as an inference from the available data from studies with mice, it seems likely that much lower uridine levels than can be achieved with parenteral uridine administration are necessary.

Additional studies of oral uridine should address the question of whether 5-FU-induced toxic effects can be abated with this mode of administration. The recommended uridine dose schedule for these studies should be 5 \(\text{g/m}^2\) every 6 hours for 3 days. Consideration could be given to adding 5-benzylacyclouridine to the uridine to increase steady-state uridine plasma levels; however, 5-benzylacyclouridine has not been used in humans thus far. For potential improvement in results, consideration could also be given to incorporating uridine in other regimens directed toward biochemical modulation of 5-FU, eg, the combination of 5-FU and leucovorin calcium \((8)\). It has already been shown in a number of clinical studies that the combination of leucovorin calcium and 5-FU is yielding response rates higher than those associated with 5-FU alone \((20)\). It is possible that adding uridine to this combination will further increase the therapeutic index of 5-FU.

References


