Modulation of Fluorouracil Toxicity With Uridine

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The antineoplastic agent 5-fluorouracil (5-FU) is used in the treatment of various tumor types. However, its antitumor activity is limited. To be active, 5-FU has to be metabolized. Its mechanisms of action have been largely elucidated but are complex. Combining 5-FU with biochemical modulating agents that interfere with 5-FU metabolism may enhance its therapeutic index. Uridine is one of these biochemical modulating agents. The aim of combining 5-FU with uridine is that the latter will reduce the toxicity of 5-FU while its antitumor activity is retained. This will enable the use of higher 5-FU doses with a potential increased antitumor effect. The combination 5-FU/uridine has shown clear activity in preclinical models. However, application in the clinic is limited. From the preclinical experience, it seemed that high doses of uridine giving rise to prolonged exposure of uridine to the tissues would be required to achieve the biochemical effect. Thus, initial clinical studies investigated tolerance and toxicities of high-dose uridine in humans. Dose-limiting toxicity was fever. High-dose uridine given as intermittent intravenous infusions was feasible and reversed 5-FU-induced leukopenia. High-dose uridine led to millimolar plasma concentrations of uridine. However, its half life was short due to rapid catabolism. Oral administration of uridine has also been studied, but bioavailability was low. Further studies are required to define the role of uridine in the biochemical modulation of 5-FU activity.

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FLUOROURACIL (5-FU) was synthesized in 1957 by Heidelberger and coworkers and is clinically used for the treatment of different tumor types, either as single agent or as part of combination chemotherapy regimens. These tumor types include breast cancer, head and neck cancer, and gastrointestinal tumors. Among the tumors of the gastrointestinal tract, colon cancer is the most common tumor type. In spite of very extensive research to develop more effective treatments, 5-FU has remained "standard" treatment for advanced stage colorectal cancer. The results, however, of this treatment are disappointing; the chance of an objective response is less than 20% without a significant influence on survival. Recently there have been indications that when 5-FU is combined with the biochemical modulator leucovorin, higher response rates are obtained. Nevertheless, despite higher response rates, a real enhancement of the therapeutic index of 5-FU in terms of significant prolongation of survival has not been achieved.

5-FU itself is inactive; in order to express a cytotoxic effect it has to be converted to one of its active metabolites, 5-fluorouridine 5'-triphosphate (FUTP), 5-fluoro-2'-deoxyuridine-5' monophosphate (FdUMP), or 5-fluorodeoxyuridine-5' triphosphate (FdUTP). FUTP is incorporated into different classes of RNA, FdUMP inhibits the enzyme thymidylate synthase (TS), resulting in decreased DNA synthesis while FdUTP may be incorporated into DNA. These events cause disruptions in DNA and RNA synthesis and are responsible for the cytotoxic action of 5-FU. In different cell lines either the DNA- or RNA-directed actions of 5-FU may be the principal determinant of growth inhibition.

BIOCHEMICAL MODULATION OF 5-FU

One concept in designing new drug combinations is to use one drug to biochemically modulate the effect of the second drug. This involves the pharmacologic manipulation of the intracellular pathways of a drug. The aim of this approach is to improve the therapeutic index. Biochemical modulation can be used to overcome 5-FU resistance. For cell culture systems and animal models, various combinations of 5-FU with other drugs have been selected on a rational basis. Examples of drug combinations based on biochemical modulation are: 5-FU and uridine, leucovorin and 5-FU, methotrexate and 5-FU, PALA and 5-FU, 5-FU and allopurinol, and 5-FU and cytidine.

Combinations of 5-FU with pyrimidines have been investigated in both preclinical models and in patients. An interesting combination is that of 5-FU and uridine chosen on the hypothesis that 5-FU antitumor activity is based mainly on inhibition of TS and side effects of 5-FU result from incorporation of 5-FU into RNA (Fig 1); if high-dose uridine is administered several hours after
5-FU, the binding ofFdUMP to TS will not be affected but uridine triphosphate (UTP) will replace FUTP in RNA. The data in Fig 2 show that uridine will cause cessation in 5-FU incorporation into RNA, while the level of 5-FU incorporated into 5-FU after 2 hours increased in samples not treated with uridine. The FdUMP binding to TS was not affected significantly in these samples. Uridine should not be given simultaneously with 5-FU because this will result in increased toxicity.

Martin et al. demonstrated successful uridine rescue in B6D2F1 mice using two different schedules of repeated injections of uridine starting 2 hours after 5-FU. Equally effective were multiple doses of uridine (800 mg/kg) on 2 successive days and a schedule of two single doses of uridine (3,500 mg/kg) separated by a period of 18 hours. In healthy mice, a single dose of 5-FU leading to about 50% mortality was rendered completely tolerable by delayed uridine treatment. Also, animals treated with 5-FU/uridine showed a lesser WBC depression compared with animals that received only 5-FU. Klubes et al. demonstrated in B6D2F2 mice rescue from lethality of 5-FU (800 mg/kg) using a 5-day subcutaneous infusion of uridine started 24 hours after 5-FU. The study by Martin et al. showed that in mice bearing tumor Colon 26, the maximal tolerated dose could be doubled with the use of delayed uridine rescue leading to enhanced antitumor activity. In a study of Peters et al., the combination of high-dose 5-FU and uridine resulted in a superior antitumor effect and increase in life span also in mice bearing Colon Tumor 26 (Fig 3). The dose of 5-FU could be increased from 100 to 250-300 mg/kg. Furthermore, in the 5-FU sensitive tumor Colon 38, the delayed administration of uridine did not affect the antitumor effect (Fig 3).

In addition, hematological toxicity of 5-FU or 5-FU plus uridine was evaluated by weekly monitoring of leucocytes, thrombocytes, and hematocrit values. With the combined 5-FU/uridine treatment, hematological toxicity was less severe than with 5-FU alone and recovery was much more rapid.

Thus, from the preclinical studies the important conclusion could be drawn that the effect of uridine was selective, i.e., it did protect against the side effects of 5-FU but did not abolish the antitumor effect.

Fig 3. Effect on life span of mice bearing Colon 26, of 5-FU alone and in combination with uridine. Life span of untreated mice was taken as control and set at 100%. Mice were treated weekly at the doses indicated (mg/kg). Uridine was administered 2 hours and 20 hours after 5-FU. Leucovorin (LV)/5-FU treatment is given as comparison.
biphasic with a mean terminal half-life of about 2 hours. The plasma concentration of the first uridine catabolite, uracil, also increased markedly with peak levels of about one tenth that of peak uridine concentrations. The plasma uracil level declined with a half-life of about 40 minutes after uridine levels had decreased to 300 μM (Fig 4).

In this 1-hour administration schedule, uridine did not protect against toxicity induced by escalated doses of 5-FU in two patients. However, this was not unexpected, because the preclinical studies clearly had shown that only prolonged exposure of the tissues to uridine protected against 5-FU-induced toxicity.

Therefore, in a further study of van Groeningen et al., prolonged IV administration of uridine was evaluated. Seven patients with advanced stage cancer participated in this study. Initially, uridine was given as continuous infusion at doses of 2.5 and 1 g/m² per hour. However, within a few hours this mode of uridine administration resulted in the occurrence of high fever and the infusions had to be discontinued. After this first experience with prolonged administration of uridine, it was felt that limiting the length of time of continuous infusion might decrease the risk of fever. Therefore, uridine infusion was further studied using an intermittent schedule. In this schedule 3-hour uridine infusions were alternated with 3-hour infusion-free intervals during a 72-hour period. Using these intermittent infusions, fever was not longer dose-limiting, mostly only mild increases in body temperature (≤1°C) were observed. The only other side effect that was ob-

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**Fig 4.** Time course of plasma concentrations of uridine (URD) and uracil (URA) after start of 1-hr uridine infusion. Representative curves for different doses are depicted and labeled to indicate the dose in g/sq m.

**Fig 5.** Representative plasma concentration versus time curves of uridine and uracil in a patient receiving uridine as an intermittent infusion at a dose of 3 g/m² per hour during 72 hours. H = 3-hour infusion period of uridine.
were administered to rabbits in a study reported by Peters et al.\textsuperscript{13} All the compounds that could be given in adequate doses induced a significant rise in body temperature. Two of these breakdown products, the secondary amino acids, carbamyl-$\beta$-alanine and $\beta$-alanine, closely resemble gamma-amino butyric acid (GABA), which plays an important role in the regulation of body temperature. An interaction between these uridine catabolites with GABA might be responsible for the observed rises in body temperature.

After it was clear that the problem of fever could be managed with the development of the intermittent uridine administration schedule, an important goal was met, i.e., the prolonged exposure of the tissues to markedly increased concentrations of the nucleoside. Therefore, with the intermittent schedule, a further study was performed evaluating the potential of uridine to protect against the toxicity of 5-FU.\textsuperscript{15} In this study, nine patients were treated with 5-FU given as weekly bolus injections. The dose of 5-FU was gradually escalated until dose limiting toxicity which was myelosuppression in all nine patients (leukopenia in five patients, thrombocytopenia in four patients). At the time of dose limiting myelosuppression, weekly 5-FU treatment was continued at the dose that produced myelosuppression. However, 5-FU was now followed after 3 hours by uridine at the dose of 2 g/m\textsuperscript{2} per hour given as intermittent IV infusions over 72 hours. As shown in Fig 6, the WBC, in the five patients developing leukopenia increased markedly, despite continued 5-FU administration. Interestingly, this effect of uridine on 5-FU-induced leu-
kopenia lasted for several weeks, although uridine was administered only after one dose of 5-FU. Also remarkably, in the four patients developing dose limiting thrombocytopenia, uridine failed to reverse this 5-FU-induced toxicity (Fig 7). The reason of this observation has yet to be explained, but this may be a mere difference in uridine metabolism in neutrophil and thrombocyte precursors. Another factor that may have affected the results is that three of the four patients who developed thrombocytopenia had received prior chemotherapy.

**ORAL ADMINISTRATION OF URIDINE**

Uridine is absorbed from the gastrointestinal tract since oral uridine is effective as replacement therapy in hereditary orotic aciduria. A major drawback of high-dose parenteral uridine administration concerns the requirement of admission to the hospital of the patients. Moreover, when uridine is administered by peripheral vein infusion, severe phlebitis of the infused vein will rapidly occur. For this reason, central venous access is necessary for prolonged administration of high-dose uridine. With respect to these drawbacks, it would be attractive to study the potential of oral uridine administration. Klubes et al reported the results of oral uridine administration in mice. Compared with parenteral uridine, the bioavailability of oral uridine was low (7%). However, prolonged and relatively constant uridine levels (33 to 82 µM) could be achieved.

A phase I clinical study on oral uridine has been performed by van Groeningen et al. As a single dose administration, the maximum tolerated dose (MTD) was 12 g/m². At the highest doses, peak plasma uridine concentrations of 60 to 80 µM were achieved. The bioavailability was low when compared with a single IV dose of uridine and amounted to 5.8% to 9.9% for dose levels of 8 to 12 g/m². To provide for more prolonged exposure of the tissues to elevated levels of uridine, the nucleoside was also administered orally every 6 hours during 3 days. In this multiple dose regimen the MTD was 5 g/m². With this dose, steady state plasma uridine levels of approximately 50 µM were achieved. Both in the single dose and multiple dose regimen, diarrhea was the dose-limiting toxicity.

It is as yet unknown what plasma uridine levels are required to protect against 5-FU-induced toxicity. Prolonged plasma concentrations in the order of 50 µM have shown to be inadequate for rescue purposes in mice by Martin et al. However, when benzylacyclouridine (BAU), an inhibitor of uridine phosphorylase, was added to uridine, and uridine levels above 70 µM could be maintained during a prolonged period, 5-FU-induced toxicity was prevented effectively.

Our preliminary data indicated that in humans oral uridine may also be effective to protect against 5-FU toxicity. In the multiple dose regimen as described above (uridine dose 5 g/m²), uridine reversed 5-FU-induced leukopenia in a small number of patients (unpublished data). However, the effect of oral uridine was clearly less marked as compared with parenteral uridine in this respect.

**CONCLUSIONS AND PERSPECTIVES**

From preclinical experiments in tumor-bearing mice it is evident that the delayed administration of uridine enhances the therapeutic index of 5-FU. In humans, it has been shown that treatment with high-dose uridine is feasible and that the nucleoside has a protective effect on 5-FU-induced myelosuppression.

The biochemical basis of this potential of uridine rescue is unclear. In different cell types, either the RNA- or DNA-directed actions of 5-FU may be the principal determinants of its cytotoxicity. Qualitative differences between tumor and normal cells may explain the ability of uridine to selectively reverse the toxic effects of 5-FU. From studies in the CD,KF, murine mammary carcinoma system, it has been concluded that the protection from 5-FU toxicity afforded by the addition of uridine is due to the reduction of 5-FU in RNA rather than by reversal of the FdUMP block on TS. It has been shown that delayed uridine administration will cause a cessation of 5-FU incorporation into RNA; this effect was already observed at a low uridine concentration of 0.1 mM (Fig 2). This last observation suggests that uridine levels that can be achieved with oral uridine may be adequate for rescue purposes.

Thus, the available evidence indicates that uridine rescue from 5-FU toxicity is related to a reduction in RNA incorporation, but a selective DNA effect might also contribute to the rescue by uridine. Sawyer et al demonstrated that DNA synthesis in bone marrow of 5-FU/uridine treated
mice recovered more rapidly than that of mice treated with 5-FU alone.

Obviously, a number of questions still have to be addressed on the potential use of uridine as a rescue agent for 5-FU toxicity. Important aspects are (1) further investigations directed to determine which level of plasma concentration is required for the biochemical and biological effects; (2) to investigate whether uridine can also protect against nonhematological toxicity that will certainly occur when myelosuppression would not be longer the dose limiting factor; (3) will the effect of uridine on 5-FU toxicity be selective in humans, i.e., will uridine abolish the antitumor effect of 5-FU? Studies in which 5-FU is combined with uridine from the start of the treatment should answer this question; and (4) will higher doses of 5-FU increase the therapeutic index of the antineoplastic agent?

Although clinical experience with uridine is as yet limited, it seems unlikely that combining 5-FU with this agent alone will improve the therapeutic index of 5-FU to a significant extent. Without doubt, the basic problem in the poor antitumor activity of 5-FU is resistance of the tumor cells to the agent. Most probably, in heterogeneous human tumors, different mechanisms of resistance exist next to each other. Using one biochemical modulating agent, only one mechanism of resistance will be dealt with. It may be that for a further improvement of the treatment, 5-FU should be combined with two or even more active biochemical modulating agents, such as leucovorin with 5-FU and uridine, which showed an improved therapeutic efficacy in mice as compared with either leucovorin/5-FU or 5-FU/uridine. Potential combinations should not only be evaluated in vitro systems, but also in in vivo animal tumor models. The results of the in vivo preclinical experiments should be translated carefully to the treatment of cancer patients. With the continuing rational development of biochemical modulation we may be successful in further enhancing the therapeutic index of 5-FU. To achieve the most optimal results, close cooperation between clinicians and laboratory investigators seems to be essential.

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