Cancer Chemother Pharmacol (1993) 32: 243–248

Cancer Chemotherapy and Pharmacology

Biochemical consequences of 5-fluorouracil gastrointestinal toxicity in rats; effect of high-dose uridine

J. Kralovanszky[1], N. Prajda[1], S. Kerpel-Fronius[1], T. Bagrij[1], E. Kiss[1], G. J. Peters[2]

[1] National Institute of Oncology, P.O.B. 21, H-1525 Budapest, Hungary
[2] Free University Hospital, Department of Oncology, De Boelelaan 1117, Amsterdam, The Netherlands

Received 5 August 1992/Accepted 3 December 1992

Abstract. Selective protection of the normal host tissues from the toxic effects of anticancer agents would allow the use of higher, probably more effective, doses of the drugs. It has been demonstrated that delayed high-dose uridine administration after 5-fluorouracil decreases the extent of myelosuppression and causes faster regeneration of the bone marrow. We studied the biochemical consequences of the gastrointestinal toxicity caused by 5-fluorouracil and the potential of high-dose uridine treatment to influence these adverse effects. 5-Fluorouracil caused dose-related decreases in the biochemical parameters (thymidine kinase, sucrase, maltase, alkaline phosphatase) selected as early markers of the impaired metabolic activity of the intestinal mucosa. The nadir of the biochemical changes was reached between 24 h and 72 h after 5-fluorouracil treatment, and complete regeneration of the mucosa took 6–7 days. Delayed high-dose uridine administration failed to mitigate the severity of the gastrointestinal damage that ensued after 5-fluorouracil treatment, but caused significantly earlier regeneration of the mucosa.

Key words: 5-Fluorouracil – Gastrointestinal toxicity – Uridine – Biochemical modulation

Introduction

5-Fluorouracil (5-FU) is widely used for the treatment of gastrointestinal (GI), breast and head and neck cancers. Severe GI and/or bone marrow toxicity limit its clinical usefulness [4].

Correspondence to: Judit Kralovanszky, Head of Clinical Research Department, National Institute of Oncology, P. O. B. 21, H-1525 Budapest, Hungary

The GI toxicity, which manifests itself as stomatitis, nausea, vomiting, diarrhea, mucositis and hemorrhage, can affect all rapidly growing epithelia of the digestive tract [1]. The extensive clinical use of 5-FU has prompted many investigations concerning the biochemistry, pharmacology, modulation and scheduling of the drug with a view to increasing its antitumor activity and decreasing its dose-limiting side effects [4, 22, 27]. In order to affect cellular growth 5-FU has to be activated to the nucleotides 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP) or 5-fluorouridine-5′-triphosphate (FUTP). FdUMP inhibits the enzyme thymidylate synthase (TS), thereby reducing de novo synthesis of dTTP and inhibiting DNA synthesis [22]. FUTP is incorporated into the RNA instead of UTP, which results in disturbances of RNA synthesis [11, 25], most probably causing processing of nuclear RNA to cytoplasmic rRNA [22].

The intracellular metabolism of 5-FU may be different in normal and in tumor cells, providing a rationale for the selective biochemical modulation of the drug [22]. It has been postulated that the gastrointestinal toxicity of 5-FU in mice is due to the incorporation of FUTP into the RNA [11], while the antitumor effect would be correlated with the level of FdUMP. These results provided a basis for the selection of uridine – a natural competitive pyrimidine – as potential rescue agent. The administration of delayed high dose uridine could rescue mice from the lethal toxic effect of 5-FU [13, 14, 17, 20]. The rescue from myelotoxicity was manifested as a more rapid recovery, both in mice [20] and man [6, 21].

Studies which evaluate the potential effect of uridine in reducing the GI toxicity of 5-FU have not been performed previously. We described a rat model to study GI toxicity induced by cytostatic agents such as cisplatinum analogues [16, 23]. For this purpose, several biochemical parameters were determined on isolated small intestinal mucosal cells. Thymidine kinase (TK) was used as a metabolic marker of the dividing crypt cells, while alkaline phosphatase (AP), sucrase (SUC) and maltase (MAL) activities were measured to characterize the digestive capacity of the matured enterocytes. These biochemical parameters are early

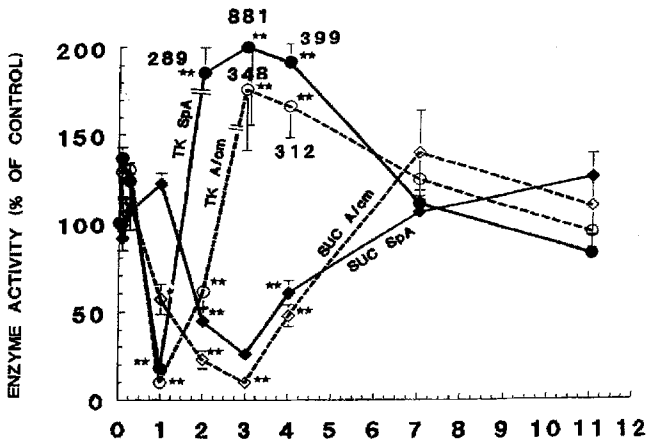

Fig. 1. Time-course of biochemical changes in TK and SUC after treatment with 5-fluorouracil. Animals were injected with 5-fluorouracil 1×83 mg/kg on day 0. Biochemical parameters were measured daily from day 1 to day 11. Enzyme activities were calculated as μmol/h per cm intestinal length (A/cm) or μmol/h per mg prot (SpA). Mean values ± SE of 4–6 rats are given and related to the enzyme activities in tissues from untreated animals. *Asterisks* denote statistically significant differences from untreated controls: $*P < 0.01$; $**P < 0.001$

markers of the impaired small intestinal functions after cytostatic treatments [16, 23]. Biochemical examinations were carried out on isolated small-intestinal mucosal cells [15]. In the present study we describe the changes in these biochemical parameters in rats treated with 5-FU in vivo. In addition we investigated the potential of delayed high-dose uridine administration in the mitigation of these side-effects.

Materials and methods

Chemicals. 5-FU was kindly provided by Hoffmann La Roche, Budapest, Hungary. Uridine was formulated as a pyrogen-free sterile solution by the Pharmacy Department and provided by the Oncology Department of the Free University Hospital Amsterdam, The Netherlands. All other chemicals were reagent grade.

Animals and treatment. Experiments were performed with healthy adult Wistar H-Riop outbred male rats weighing 180–200 g. Animals were kept in controlled conditions and had access to food and water ad libitum. The rats received 5-FU and uridine treatment as i.p. bolus injections. The injection volume was 0.01 ml/g of body weight. Doses and dosage schedules are given in the legends of each figure and table. Control animals received 0.9% NaCl instead of drugs. In each group 4–6 animals were treated.

Isolation of intestinal cells. Animals were killed 24 and 48 h after 5-FU administration in the dose-response studies. For the study of time course rats were sacrificed at 6, 24, 48, 72, 96, 168 h after the 5-FU injection. The small intestine was removed after the Treitz ligament and washed with 154 mM NaCl containing 1 mM mercaptoethanol. Intestinal epithelial cells were isolated with a combined chemical mechanical method described in detail earlier [15, 28].

Biochemical determinations. Isolated cells were centrifuged for 10 min at 1500 rpm (500 *g*) at +5° C, washed with isotonic KCl and homogenized in isotonic KCl. Subsequently the homogenate was centrifuged for 60 min at 100 000 *g*. For the determination of AP, SUC an MAL activities the whole homogenate and for that of TK the 100 000 *g* supernatant was used.

AP activity was determined with *p*-nitrophenylphosphate as substrate, and the *p*-nitrophenol released was measured spectrophotometrically at 405 nm [2]. Disaccharidase activities were estimated by measuring the rate of hydrolysis of SUC and MAL, using the glucose oxidase-peroxidase method to measure the amount of glucose formed [3]. TK activity was measured by determining the conversion of 2-[^{14}C]-TdR to 2-[^{14}C]-TMP by the DEAE cellulose disc method [12]. Protein content was determined by the method of Hartree [9]. AP, SUC and MAL activities were calculated as micromoles per hour per centimeter of intestine, and that of TK as nanomoles per hour per centimeter of intestine. Statistical analysis was performed by using Student's *t*-test for unpaired samples.

Plasma uridine concentrations. Blood samples of rats were taken at several time points in heparinized tubes following i.p. uridine administration. After centrifugation plasma samples were frozen and stored at −20° C. After thawing, these samples were extracted with 5% trichloroacetic acid as described previously [19] and were analysed for uridine and uracil using a standard reversed-phase HPLC method [19].

Results

General observations

5-FU given as a single i.p. dose (21–250 mg/kg) caused serious side-effects in the GI tract of rats. Diarrhea began as soon as 48 h after the treatment and lasted 5–6 days. With increasing dose there was an increase in weight loss. The LD_{50} value of 5-FU for the H-Riop Wistar outbred male rats was 250 mg/kg. The individual days of death between the 1st and 9th days after the treatment were indicative rather of GI than of bone marrow toxicity.

In vitro effect of 5-FU on the intestinal marker enzymes

To determine whether 5-FU itself affected the activity of the marker enzymes, 5-FU was added directly to the enzyme assays in concentrations of 0.1 mM–10 mM. In this concentration range, 5-FU did not cause significant changes in the enzyme activities.

Time course of biochemical changes after treatment with 5-FU

Before viewing the time course of biochemical changes after a single 83 mg/kg dose of 5-FU (Fig. 1), it must be remembered that intestinal cells are not made up of a single population but there is a differentiation gradient from the crypt to the villus region. Therefore, we demonstrate changes in activity of TK, a marker of mitotically active cells, and that of SUC, which is an excellent marker of the differentiated villus cells.

The activity profiles of the two enzymes were very similar either the results were expressed per centimeter of intestinal length or per milligram of protein. This means that the enzyme activity decrease and increase observed after 5-FU treatment not only reflected the change in cellularity and repopulation but was probably connected with a

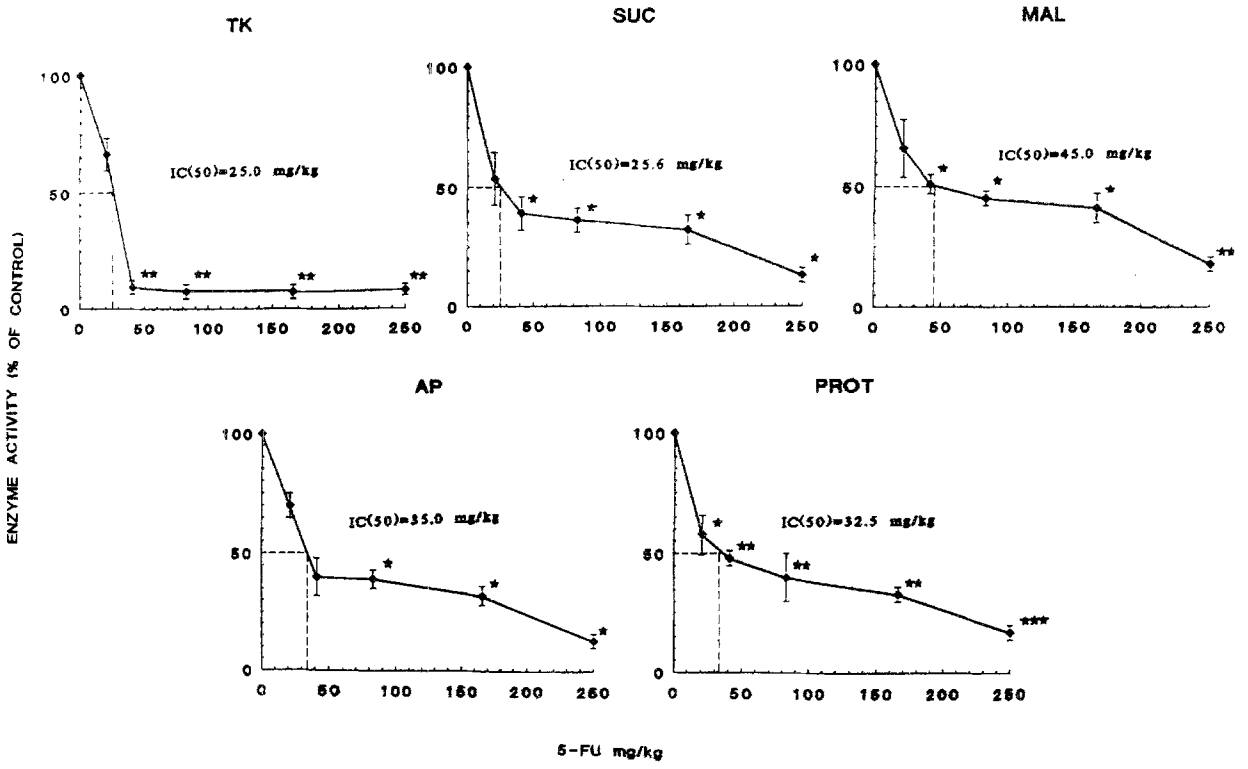

Fig. 2. Dose-response curves for biochemical parameters in isolated rat intestinal mucosal cells after 5-fluorouracil treatment. Rats were treated with a single dose (21–250 mg/kg) of 5-fluorouracil. The TK activity change was determined 24 h and that of the other parameters 48 h after the treatment. Mean values ± SE of 4–6 rats are given. *Asterisks* denote statistically significant differences from untreated controls: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$

decrease in enzyme concentration per cell caused by the disturbed cell differentiation.

The distribution pattern of TK was different from that of SUC. In the first 6 h after the treatment no change or a very slight increase of the enzyme activities was observed. Thereafter a sharp decrease of TK activity started, reaching the activity nadir as soon as 24 h after the treatment. This was followed by a very sharp rise in the enzyme activity as a consequence of the compensatory increased proliferative activity of the dividing cell zone. TK activity reached the normal level, followed by a rebound between 48–72 h.

The decrease in the activity of SUC started and developed later and the maximal lesion was observed between 48–72 h after 5-FU administration. Complete biochemical regeneration of the mucosa took 6–7 days. The other two functional enzymes of the matured enterocytes, AP and MAL, showed a similar pattern to that of SUC. (Data not shown).

Dose-response studies with various doses of 5-FU

The dose-response relation of the biochemical parameters on 5-FU was investigated after different single doses of the drug 24, and 48 h after the treatment (Fig. 2). The results show that in the dose range examined (21–250 mg/kg) the decreases in all biochemical parameters studied were directly related to the 5-FU doses. The enzyme activities decreased sharply even with the lowest dose of 5-FU (21 mg/kg), and the levels were then significantly different from those in the untreated controls in the dose range of 43–250 mg/kg 5-FU. The values of the inhibitory constants (I_{50}) calculated from the dose-response curves were in the therapeutic dose range, showing that during 5-FU treatment about a 50% decrease in the metabolic activity of the intestinal mucosa cells should be expected.

Effect of schedule on the intestinal toxicity of 5-FU

To study the effect of the schedule on the GI toxicity of 5-FU, we compared the biochemical changes in the intestinal mucosal cells after treating the animals either with a single high dose (1×84 mg/kg) or with repeated small doses (4×21 mg/kg daily) of 5-FU. For comparison we also measured the marker enzymes after treatment with a single 1×21 mg/kg dose of 5-FU. Rats from all the three groups together with the untreated controls were killed 24, 48 and 96 h after the single doses or after the last dose of repeated 5-FU treatment (Fig. 3).

For the functional enzymes (AP, SUC, MAL) no significant difference ($P > 0.05$) in the extent of decrease in enzyme activity at the time of nadir was observed between the groups treated with single high dose and repeated small doses of 5-FU. However the nadir was 24 h earlier in the latter group. In the animals which received repeated small doses, TK activity did not show the rapid and marked decrease observed for the single high dose. Probably the

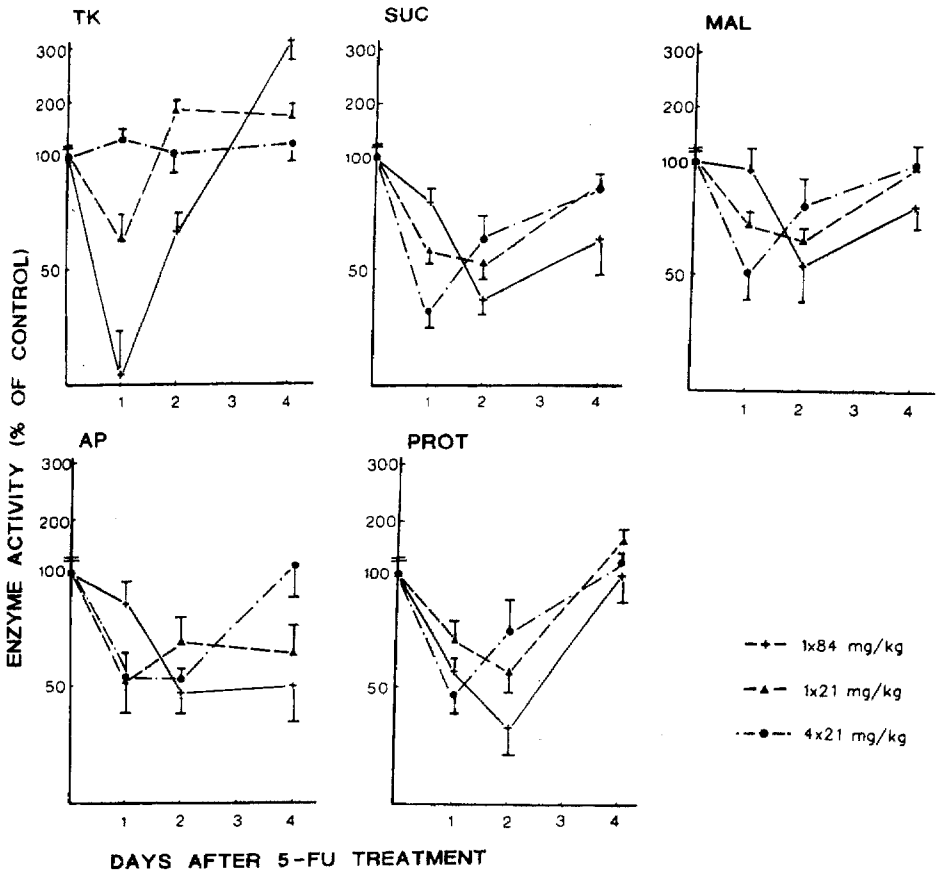

Fig. 3. The effect of dosage schedule on the small intestinal toxicity caused by 5-fluorouracil. Animals were treated with a single high dose (1 × 84 mg/kg), divided small doses (4 × 21 mg/kg) given daily or with a single small dose (1 × 21 mg/kg) of 5-fluorouracil. Biochemical determinations were performed 24, 48 and 96 h after the single doses or after the last dose of 5-fluorouracil. Mean values ± SE of 3–5 animals are shown

rapid increase in TK activity seen 48 h after the first dose of 5-FU compensated the decreases caused by the subsequent 5-FU doses.

The time required for biochemical regeneration of the mucosa was shorter in the group treated with 4 × 21 mg/kg 5-FU than in the animals which received the single high dose of 5-FU.

Effect of delayed high-dose uridine treatment on the small-intestinal toxicity induced by 5-FU

We examined the possibility of mitigating the GI toxicity of 5-FU by delayed high-dose uridine administration. Animals were treated with 5-FU (1 × 83 mg/kg) alone or in combination with uridine 3.5 g/kg administered 2 and 20 h after 5-FU. The changes in enzyme activity were studied 1, 2, 3, 4 and 7 days after the 5-FU injection.

Uridine administration failed to reduce the toxic effect of 5-FU on the small intestinal mucosal cells. The extent of the decrease in enzyme activity in the group treated with 5-FU alone was similar to that obtained in the group treated with 5-FU + uridine (Fig. 4). However, there was a more rapid recovery of the enzyme activities to the pretreatment levels in the uridine-treated groups. The data presented in Fig. 4 suggest that complete regeneration of the intestinal mucosa was achieved earlier if the same dose of 5-FU was administered in combination with delayed high-dose uridine.

Plasma uridine concentration

Plasma plateau level of uridine after i.p. administration were in the same range as observed previously in mice [19] about 5 mM (up to 2 h after administration of 2 g/kg) and about 20 mM (up to about 1 h after administration of 3 g/kg). Plasma uracil levels varied between 2 and 3 mM during this time period.

Discussion

5-FU and other fluorinated pyrimidine derivatives cause different lesions in the gastrointestinal system of experimental animals but so far neither histological nor biochemical investigations have made it possible to identify the specific change(s) causing the death of the experimental animals [18].

5-FU given in a single dose produced dose-related reversible lesions in the small intestinal mucosa even at the therapeutic dose. This was manifested in the decrease of most enzyme activities which were important for the normal cell proliferation and for the digestive function of the intestinal mucosa. The capability of intestinal cells to hydrolyze disaccharides to glucose and galactose is decreased, and the undigested, osmotically active disaccharides pass through the small intestine into the colon, where they extract water, causing diarrhea [18].

In the first 24 h after the 5-FU injection only the dividing crypt cells were damaged, judging by the low TK and

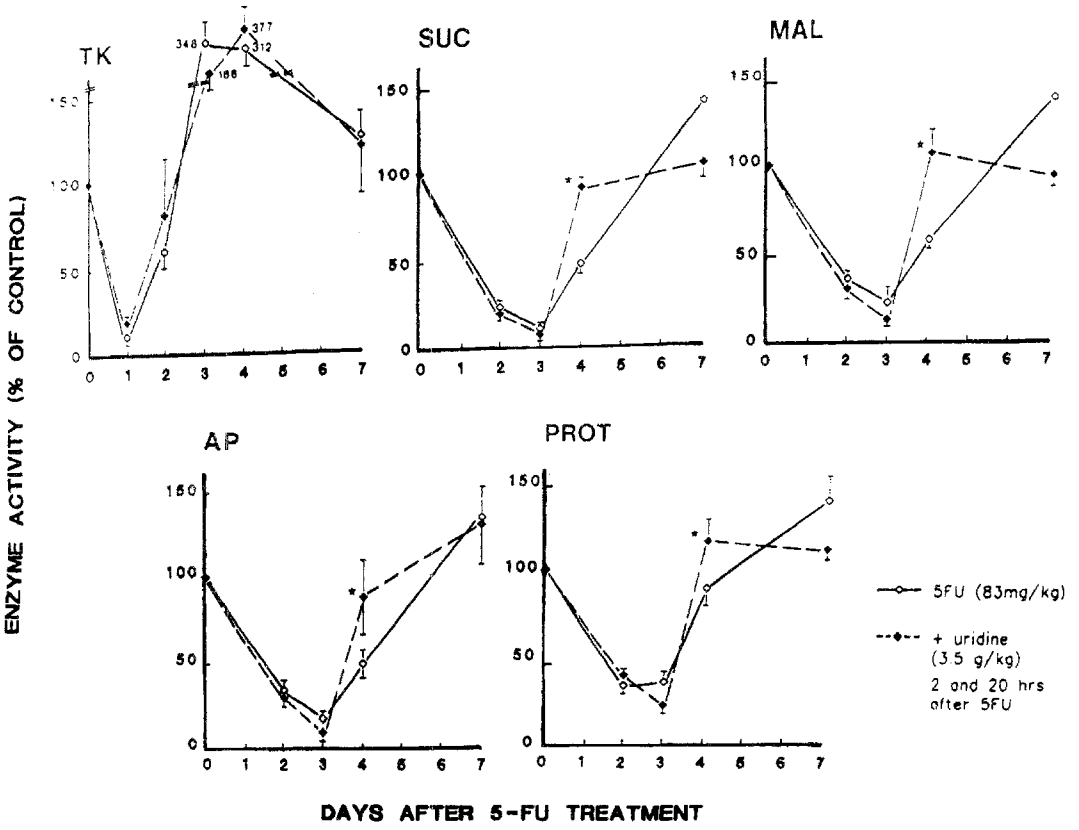

Fig. 4. Comparison of the time courses of biochemical changes obtained after treatment either with 5-fluorouracil alone or with 5-fluorouracil + high-dose uridine. Group of 8–10 rats were injected with 5-FU (83 mg/kg) on day 0. After treatment animals were divided into two groups with 4–5 animals in each. The first group received uridine 3.5 g/kg per day, 2 and 20 h after 5-FU treatment, second group received 0.9% NaCl instead of uridine. Biochemical investigations were performed on days 1–7 in both groups. Mean values ± SE of 4–5 animals are given. *P <0.05 (statistically significant difference from the value obtained in the 5-FU treated group)

almost intact brush border enzyme activities. The use of TK as a marker for toxicity has a complicating factor when evaluating the effects of 5-FU. TK is an important enzyme in the supply of thymidine nucleotides via the salvage pathway. However, the rate-limiting de novo enzyme, thymidylate synthase, is also severely inhibited in the intestinal mucosa of the rat [26]. This inhibition is retained for a longer period. A prolonged depletion of thymidine nucleotides will probably lead to enhanced GI toxicity. Functional enzymes were only slightly affected until 48 h, but thereafter the decrease was long-lasting and complete regeneration of the intestinal function took 6–7 days. Similar, but less serious biochemical changes and more rapid regeneration were demonstrated after treatment with cisplatin and iproplatin, whereas carboplatin treatment caused only very mild intestinal toxicity [16].

Since 48 h are required for the production of mature enterocytes [10], the time course of the damage of the brush-border enzymes supports the proposition that action of 5-FU on these biochemical processes is indirect in nature. Inhibition of mitotic activity, which became manifest very early within the first 24 h, was demonstrated by Roche et al. [24]. The maximal lesion appeared on the 3rd to 4th days, when the glucose absorption was reduced significantly and the digestive enzyme activities decreased [10, 24]. These data fit well with the differences in the side-effects of 5-FU observed in clinical practice. The dose-limiting toxicity of weekly bolus 5-FU is not GI toxicity but myelotoxicity. However, for daily bolus injections and continuous infusions GI toxicity is predominant (including diarrhea and mucositis). The very low I_{50} values of the intestinal marker enzymes, which were within the therapeutic dose range, demonstrated that GI toxicity might be dose limiting with this schedule. Biochemical modulation can be useful to improve the therapeutic index of 5-FU either by enhancing its antitumor effect or by decreasing its toxicity against the normal tissues.

Two biochemical mechanisms are supposed to play a role in the protection of 5-FU toxicity by uridine. First, uridine competes with 5-FU for incorporation into RNA. Secondly, the inhibition of TS by FdUMP will be disturbed by the elevated amount of dUMP. Results of Sawyer et al. [25] showed that 1000 mg/kg uridine could not prevent the inhibition of TS by FdUMP, supporting the hypothesis that the decreased 5-FU incorporation into RNA is more probably responsible for the protective effect of uridine.

As for the schedule of uridine administration, it was found that similar bone marrow protection could be achieved in mice after high-dose bolus uridine (3500 mg/kg) treatment or after repeated small doses (800 mg/kg every 2 h for 4 doses) [17]. In patients the rescue by uridine was schedule-dependent. Bolus injec-

tions and continuous infusions would not be applied, while an intermittent uridine schedule resulted in reversal of hematological toxicity [5, 6]. Currently oral uridine is under investigation [7]. Plasma levels obtained in mice in vivo, as against in vitro results, were high enough to effect rescue in the presence of 5-FU toxicity [20]. The plasma levels of uridine observed in the rat were similar to those observed in mice [19]. In the case of small-intestinal mucosa the 5-FU-induced biochemical changes were not milder after delayed high-dose uridine administration, but recovery was faster. Limited rescue in the GI tract by uridine was established.

Conclusion

5-FU caused severe dose-dependent decreases in the biochemical parameters (thymidine kinase, sucrase, maltase, alkaline phosphatase) selected as early markers of the small intestinal toxicity. The I_{50} values of the enzymes were in the therapeutic dose range. Intermittent high-dose uridine administration failed to mitigate the severity of the GI damage, but was followed by earlier regeneration of the small-intestinal mucosa.

Acknowledgements. The authors wish to acknowledge the technical assistance of Judit Kutvölgyi and the secretarial help of Judit Bódi. The work reported in this paper was supported in part by grant 3024 OTKA from the Hungarian Academy of Sciences and in part by grant T-415/1990 ETT from the Ministry of Welfare, Hungary.

References

1. Bateman JR (1971) 5-Fluorouracil given once weekly. Comparison of intravenous and oral administration. Cancer 28: 907
2. Bessey OA, Lowry OH, Brock MI (1965) Determination of alkaline phosphatase activity in serum with *p*-nitrophenylphosphate. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Verlag Chemie, Weinheim/Academic Press, New York London, p 783
3. Dahlquist A (1968) Assay of intestinal disaccharidases. Anal Biochem 22: 99
4. Grem JL (1990) Fluorinated pyrimidines. In: Chabner B. H., Collins F. M. (eds) Cancer chemotherapy. Principles and practice. Lippincott, Philadelphia, p 180
5. Groeningen CJ van, Leyva A, Kraal I, Peters GJ, Pinedo HM (1986) Clinical and pharmacokinetic studies of prolonged administration of high dose uridine intended for rescue from 5-FU toxicity. Cancer Treat Rep 70: 745
6. Groeningen CJ van, Peters GJ, Leyva A, Laurensse E, Pinedo HM (1989) Reversal of 5-fluorouracil induced myelosuppression by prolonged administration of high dose uridine. J Natl Cancer Inst 81: 157
7. Groeningen CJ van, Peters GJ, Nadal JC, Laurensse E, Pinedo HM (1991) Clinical and pharmacologic study of orally administered uridine. J Natl Cancer Inst 83: 437
8. Harrison SD, Denine EP, Peckham JC (1978) Qualitative and quantitative toxicity of single and sequential sublethal doses of 5-fluorouracil in BDF_1 mice. Cancer Treat Rep 62: 533
9. Hartree EF (1970) Determination of protein. A modification of the Lowry method which gives a linear photometric response. Anal Biochem 48: 422
10. Hartwich G (1974) Side effects of a cytostatic treatment on the gastrointestinal tract. Acta Hepatogastroenterol 21: 89
11. Houghton JA, Houghton PJ, Wooten PS (1979) Mechanism of induction of gastrointestinal toxicity in the mouse by 5-fluorouracil, 5-fluorouridine and 5-fluoro-2′-deoxyuridine. Cancer Res 39: 2406
12. Klemperer C, Haynes J (1968) Thymidine kinase in rat liver during development. Biochem J 108: 541
13. Klubes P, Cerna I (1983) Use of uridine rescue to enhance the antitumor selectivity of 5-fluorouracil. Cancer Res 43: 3182
14. Klubes P, Cerna I, Meldon MA (1982) Uridine rescue from the lethal toxicity of 5-fluorouracil in mice. Cancer Chemother Pharmacol 8: 17
15. Kralovanszky J, Prajda N, Kerpel-Fronius S, Szentirmay Z (1981) Biological model system for investigating gastrointestinal side effects caused by cytostatic agents. In: Mòzsik G., Hänninen O., Jàvor T. (eds) Gastrointestinal defence mechanisms. Pergamon Press London/Akademiai Kiado, Budapest, p 327
16. Kralovanszky J, Prajda N, Kerpel-Fronius S, Gal F, Kiss F (1988) Comparison of intestinal toxic effects of platinum complexes: cisplatin (CDDP) carboplatin (CBDCA) and iproplatin (CHIP). Cancer Chemother Pharmacol 21: 40
17. Martin DS, Stolfi RL, Sawyer RC, Spiegelman S, Young CW (1982) High dose 5-fluorouracil with delayed uridine "rescue" in mice. Cancer Res 42: 3964
18. Mózsik Gy, Snodgrass W (1986) Clinical gastrointestinal toxicology: In: Rozman K (ed) Gastrointestinal toxicology. Elsevier, Amsterdam New York Oxford, p 436
19. Peters GJ, Groeningen CJ van, Laurensee EJ, Lankelma J, Leyva A, Pinedo HM (1987) Uridine induced hypothermia in mice and rats in relation plasma and tissue levels of uridine and its metabolite. Cancer Chemother Pharmacol 20: 101
20. Peters GJ, Van Dijk J, Laurensse E, Groeningen CJ van, Lankelma J, Leyva A, Nadal JC, Pinedo HM (1988) In vitro biochemical and in vivo biological studies of the uridine rescue of 5-fluorouracil. Br J Cancer 57: 259
21. Peters GJ, Groeningen CJ van (1991) Clinical relevance of biochemical modulation of 5-fluorouracil. Ann Oncol 2: 469
22. Pinedo HM, Peters GJ (1988) Fluorouracil: biochemistry and pharmacology. J Clin Oncol 6: 1653
23. Prajda N, Kralovanszky J, Kerpel-Fronius S, Gal F, Szentirmay Z (1985) Enzymological and morphological changes in rat intestinal mucosa following treatment with alkylating sugar alcohol derivatives. Anticancer Res 5: 451
24. Roche AC, Bognel JCI, Bognel C, Bernier JJ (1970) Correlation between the histological changes and glucose intestinal absorption following a single dose of 5-fluorouracil. Digestion 3: 195
25. Sawyer RC, Stolfi RL, Spiegelman S, Martin DS (1984) Effect of uridine on the metabolism of 5-fluorouracil in the CD_8F_1 murine mammary carcinoma system. Pharmacol Res 2: 69
26. Van de Wilt CL, Marinelli A, Smid K, Cloos J, Pinedo HM, Peters GJ (1992) Inhibition of thymidylate synthase (TS). In normal tissues a toxic side effect of 5-fluorouracil (5FU). Proceedings of The 83rd Annual Meeting of the American Association for Cancer Research, abstract 2536
27. Weckbecker G (1991) Biochemical pharmacology and analysis of Fluoropyrimidines alone and in combination with modulators. Pharmacol Ther 50: 367
28. Weiser MM (1973) Intestinal epithelial cell surface membrane glycoprotein synthesis. J Biol Chem 248: 2536