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Review paper

5-Fluorouracil in colorectal cancer: rationale and clinical results of frequently used schedules

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Colorectal cancer is one of the most frequent cancers in the western world. Approximately half of the patients will die of their disease because of metastases. The most active cytotoxic agent used to date is 5-fluorouracil (5-FU). However, clinical responses are achieved only in a minority of patients. Based on the current knowledge of the mechanism of action of 5-FU, many attempts have been made to improve the clinical results. These include the use of biochemical modulators and different methods of administration, and these are the subject of this review. Specifically, of five different modulators, i.e. leucovorin, methotrexate, interferon -α, *N*-(phosphonacetyl)-L-aspartate and trimetrexate glucuronate, the biochemical background and the clinical results obtained with these modulators are discussed. In order to get more insight, an overview of the 5-FU metabolism has been given. In addition, the different methods of systemic administration of 5-FU as well as possible mechanisms underlying 5-FU resistance are described. [© 1998 Lippincott-Raven Publishers.]

Key words: 5-Fluorouracil, biochemical modulation, clinical trials, metabolism.

Introduction

The prognosis of metastatic colorectal cancer is dismal. The median survival of advanced colorectal cancer is approximately 6 months for untreated patients. Since its synthesis in 1957 by Heidelberger, 5-fluorouracil (5-FU) is still the most effective drug in metastatic disease.[1] Response percentage of 5-FU-containing regimens varies between 10 and 30%. The response rate has been improved in recent decades by the use of various biochemical modulators. However, the question remains if this may be ascribed to improvement of the modulation, because in most randomized studies modulated 5-FU was not compared with equitoxic doses of unmodulated 5-FU. The modulators that are discussed in this review are leucovorin (LV), methotrexate (MTX), interferon (IFN)-α, *N*-(phosphonacetyl)-L-aspartate (PALA) and trimetrexate glucuronate (TMTX) (Figure 1). The modulators have been used in clinical trials during the past decade. Recently developed modulators with promising activity are mentioned briefly. Preclinical research has also resulted in the exploration of different methods of 5-FU administration. We will discuss bolus injection, continuous infusion and chronomodulation. Information on possible mechanisms underlying 5-FU resistance is reviewed.

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Mechanism of action of 5-FU

5-FU is metabolized into active anabolites, in particular in tumor cells, and into inactive catabolites, especially in the liver, but also in extrahepatic tissue.[2-4] 5-FU itself is rapidly cleared from the plasma within 15-20 min, but its metabolites may persist in the tissues for several days.[5]

The scheme of 5-FU metabolism is presented in Figure 1. Only a small percentage (1-3%) of the total body dose of 5-FU is converted to the therapeutically relevant anabolites: the fluoronucleosides (FNucs) and fluoronucleotides (FNuct).[6] The most important FNuct for the cytotoxic effect of 5-FU are 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP) and 5-fluorouridine-5'-triphosphate (FUTP), the latter generally occurring at a much higher concentration.[7] Initial steps in the anabolic metabolic pathways of 5-FU are its conversion to the FNucs 5-fluorouridine (FUrd) and 5-fluoro-2'deoxyuridine (FdUrd), and to 5-fluorouridine-5'-monophosphate (FUMP). The latter can be

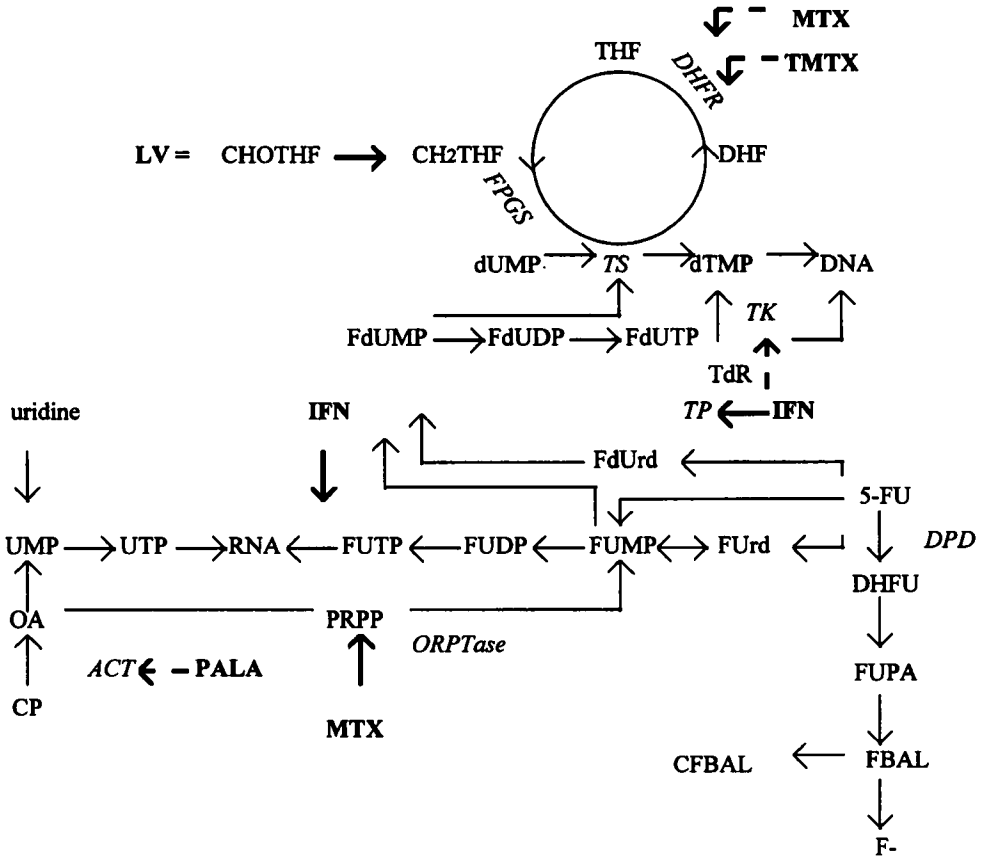

Figure 1. Diagram of the interaction between 5-FU and pyrimidine metabolism. Modulators are printed bold, enzymes in italics; solid arrows, stimulation; dashed arrows, inhibition; *ATC*, aspartate transcarbamylase; *OPRTase*, orotate phosphoribosyltransferase; *TP*, thymidine phosphorylase, *TK*, thymidine kinase; *DPD*, dihydropyrimidine dehydrogenase; *DHFR*, dihydrofolate reductase; *FPGS*, folylpolyglutamate synthase. The ternary complex responsible for TS inhibition is represented by a circle. Abbreviations other than used in the manuscript: CP, carbamyl phosphate; OA, orotic acid; TdR, thymidine; F-, unknown fluor combinations.

constituted from FUrd, but is mainly formed directly via the orotate phosphoribosyltransferase (OPRTase) enzymatic system in the presence of the co-substrate 5′-phosphoribosyl-1-pyrophosphate (PRPP). FUMP is further converted either to FUDP and then to FUTP via a kinase system, or to FdUMP via the ribonucleotide reductase enzymatic system, and then to the di- and triphosphate derivates (FdUDP and FdUTP).[8] A large fraction (80–85%) of the administered 5-FU is degraded to catabolites, i.e. 5′,6′-dihydro-5-fluorouracil (DHFU), α-fluoro-β-ureidopropionic acid (FUPA) and α-fluoro-β-alanine (FBAL).[2,6] Between 5 and 20% of 5-FU is excreted unchanged in the urine.

Three major mechanisms underlying the antitumor activity of the FNuct have been suggested and the relevance of these mechanisms is still a matter of considerable debate.[1,9] First, inhibition by FdUMP of the key enzyme of pyrimidine *de novo* synthesis, thymidylate synthase (TS). This enzyme catalyzes the conversion of dUMP to dTMP and its inhibition results in decreased DNA synthesis. Second, incorporation of FUTP into RNA, yielding fraudulent RNA. Third, incorporation of FdUTP into DNA.[8] However, the extent to which the last mentioned 5-FU incorporation into DNA relates to 5-FU cytotoxicity is unclear. It is generally agreed upon that the FNuct formed as a result of anabolism are responsible for the therapeutic effect of 5-FU.

Catabolism of 5-FU to FUPA and FBAL in normal tissues is a detoxification mechanism.

In order to increase the ratio of anabolism to catabolism, biochemical modulators have been used. Biochemical modulation involves the use of one or more agents, which themselves may be devoid of

cytotoxic activity, but which enhance the antitumor effect or the selectivity of an active antitumor drug by altering the biochemical pathways involved.[10] Ideally the effect of the modulators on 5-FU metabolism should be more pronounced in tumor tissue than in normal tissues, but unfortunately this is not the case. For 5-FU, several modulators of its antitumor activity have been reported, although the actual effect of the different modulators on 5-FU metabolism has not been unambiguously demonstrated for all of these modulators.[11]

Biochemical modulators of 5-FU

Multiple factors are associated with the sensitivity of tumor cells to 5-FU. These include the presence of co-substrates (e.g. PRPP), the activity of anabolic enzymes (e.g. OPRTase), the presence of competing nucleosides (e.g. uridine) and nucleotides (e.g. deoxythymidine triphosphate [dTTP]), the kinetics of the catabolism of 5-FU, the extent of conversion of 5-FU into FUMP and FdUMP, and alterations in the amount of TS present (e.g. gene amplification).[12] Biochemical modulation of 5-FU activity may be accomplished by interfering with each of these determinants in order to improve the therapeutic index. This concept has been most extensively tested in the treatment of human colorectal cancer.[3,9,11]

Specific exposure protocols might be required for the *in vivo* biochemical modulation of 5-FU activity. Thus, differences in the sequence and time schedule of administration of the two agents (5-FU and its modulator) may contribute to the different results of clinical trials.

LV

LV increases the stability of the FdUMP-TS-N^5,N^{10}-methylenetetrahydrofolate ternary complex. This ternary complex is formed because FdUMP, one of the metabolites of 5-FU, binds to TS in the presence of N^5,N^{10}-methylenetetrahydrofolate (CH_2THF). This interaction leads to the formation of a covalent ternary complex and to the inhibition of TS. Thus FdUMP acts as a suicide inhibitor for TS, forming a covalent ternary complex with the enzyme in combination with the cofactor CH_2THF.[11,13] The degree of inhibition of TS and the persistence of this inhibition are essential factors for maximal growth inhibition by 5-FU.[9] Administration of 5-FU together with LV results in an increase in the intracellular concentration of CH_2THF and in stabilization of the ternary complex.[14] As a consequence, the essential nucleotides for the synthesis of DNA (dTTP) are depleted, eventually resulting in cell death.[2,15] In addition to this role in TS inhibition, FdUMP can be converted to FdUTP and incorporated into DNA, resulting in strand breaks.[16]

MTX

MTX causes an increase of the intracellular pool of PRPP through inhibition of the *de novo* purine synthesis. The increased PRPP concentration results in increased conversion of 5-FU to FUMP by OPRTase, which uses PRPP as a co-substrate. Increased formation of FUMP results in an increased formation of FUTP with increased probability of the incorporation of FUTP instead of UTP into RNA, causing cytotoxicity.[2,17] Moreover, MTX is a potent competitive inhibitor of dihydrofolate reductase (DHFR). This enzyme is required for the regeneration of tetrahydrofolate (THF), which is essential for the synthesis of dTMP.[13] Inhibition of dTMP synthesis adversely affects DNA synthesis.

IFN-α

IFNs induce changes in the pharmacokinetics of 5-FU. However, the exact mechanism of the interaction between IFN-α and 5-FU is still unknown. IFN-α is reported to reduce the plasma clearance of 5-FU and to enhance the incorporation of 5-FU into RNA.[18,19] IFN-α has been found to increase the expression of the enzyme thymidine phosphorylase (TP), which catalyzes the first step in the conversion of 5-FU to the active metabolite FdUMP in human colon carcinoma cells.[19,20] The formation of FdUMP plays a central role in 5-FU therapy through inhibition of TS as described above.[21] It has also been observed that IFNs reduce cellular uptake of thymidine and reduce the activity of the enzyme thymidine kinase (TK). In this way the normal biochemical role of thymidine in nucleic acid synthesis, necessary for cell replication, is disrupted.

PALA

PALA has been reported to inhibit aspartate transcarbamylase (ATC), a key enzyme of the *de novo* pyrimidine biosynthesis, by competing with its natural substrate, carbamyl phosphate.[9,22] This leads to an increase in PRPP, a depletion of orotic acid (the precursor of UMP), and the depletion of uracil and cytosine nucleotides (UMP, UDP, UTP and CTP) and pyrimidine deoxyribonucleotides (dUMP, dUTP and dTTP).[11] A decreased dUMP concentration causes decreased competition

with FdUMP for TS binding. The increased availability of PRPP can be expected to favor the formation of FUMP via OPRTase as already outlined above.[2,23-25]

TMTX

TMTX is a non-classical quinazoline antifol that lacks a terminal glutamic acid residue. Like MTX, TMTX competitively inhibits dihydrofolate reductase and increases intracellular concentrations of PRPP.[26,27] Unlike MTX, TMTX is lipophilic and appears to enter cells by passive diffusion. It does not undergo intracellular polyglutamation and it does not require the reduced folate transport system, and therefore does not compete with LV for cellular uptake and metabolism.[28,29]

^{19}F NMR

To study the effectivity of a combination of 5-FU with a modulator we use ^{19}F NMR spectroscopy. With this method it is possible to detect 5-FU and its metabolites in fluids and in living tissues. We use ^{19}F spectroscopy to investigate the 5-FU metabolism *in vitro* and *in vivo* in rodents, and recently also *in vivo* in patients with advanced colorectal cancer receiving 5-FU treatment. In our study of tumor extracts of murine colon tumors treated with 5-FU and different modulators studied with ^{19}F NMR spectroscopy *in vitro*, we concluded PALA was not effective as was later approved by the clinical study.[30]

Methods of 5-FU administration

5-FU is usually administered as a bolus, i.e. short-term infusion. The importance of administering 5-FU in bolus schedules, indeed as short-term infusions, is illustrated by the following studies. In a retrospective non-randomized study patients receiving a 5-FU bolus injection within 2 min had a higher response rate compared to patients receiving the same 5-FU dose in 20 min; 32 versus 10%, respectively ($p<0.001$). In spite of more toxic side effects, more subjective improvement was noted in the former group of patients.[31] In a prospective randomized trial bolus 5-FU administration, given as an injection during 2-4 min, was compared with short-term infusion lasting for 10-20 min, both combined with LV.[32] In the first group 27% responses were seen versus 13% in the second group (p=0.02), whereas both groups showed no difference in toxicity and long-term survival.

Because 5-FU is an antimetabolite and therefore only active in dividing cells and because colorectal cancer is a slowly growing tumor, a longer exposure time should result in the uptake of 5-FU by more tumor cells. With continuous infusion a higher dose intensity can be reached. In continuous infusion schedules 5-FU is usually infused over 24 or 48 h or protracted for several weeks. Bolus injections and continuous infusion are thought to have different mechanisms of cytotoxicity and of resistance. Administration of 5-FU as a bolus injection produces high plasma levels of 5-FU and increased FUTP incorporation into RNA, whereas continuous infusion results in low plasma levels of 5-FU and lower RNA incorporation but prolonged TS inhibition.[33] Partial response and disease stabilization have been obtained with continuous infusion in patients who were in progression after bolus treatment, supporting the experimental finding that resistance to bolus 5-FU may be overcome by continuous infusion 5-FU.[34] Bolus injections and continuous infusion have been alternated in order to overcome resistance.[35]

Although continuous infusion generally increases the response rate compared with bolus injections, there is no big difference in overall survival.[36] However, there is a different toxicity pattern with stomatitis being more severe after continuous infusion and leukopenia after bolus injection.[37,38] A popular regimen is the combination of bolus injection and continuous infusion.[39] In a randomized trial this regimen was superior to monthly low-dose LV and 5-FU bolus in terms of response rate and toxicity, but not in survival. In a meta-analysis, which involved six randomized trials of continuous infusion 5-FU versus bolus 5-FU, a significantly higher response rate (22 versus 14%, respectively) and median survival (12.1 versus 11.3 months, respectively) was demonstrated in favor of continuous infusion 5-FU, although the median survival times were close.[36]

Circadian timing of drug delivery (chronomodulation) is a method to further improve dose intensity compared with protracted infusion at a flat rate. This is based on observations on circadian changes in the enzymatic activities involved in 5-FU catabolism and anabolism as well as in the proliferative activity of target tissues of toxicity (i.e. bone marrow and mucosa). Programmable-in-time pumps allow to test the clinical relevance of this principle.

Mechanisms underlying 5-FU resistance

As is pointed out in the Introduction, response rates to 5-FU treatment in advanced colorectal cancer vary

between 10 and 30%. Virtually all patients eventually develop resistance to 5-FU. Three possible mechanisms of resistance have been observed and will be described here.

The first mechanism underlying 5-FU resistance occurs at the level of TS inhibition. The relevance of both pre- and post-treatment TS activity or expression has been demonstrated. As to pre-treatment TS activity, intratumoral ratios of TS/β-actin mRNA for tumor biopsies of disseminated colorectal cancer patients have been determined and the median TS/β-actin ratio was found to significantly segregate responders (low ratio) from non-responders (high ratio).[40] Also median survival was longer for patients with a low ratio. Furthermore, intratumoral TS levels were correlated with overall survival.[41] The importance of the post-treatment 5-FU-induced increase of TS and of the duration of TS inhibition has been demonstrated. High TS levels and low inhibition of TS upon 5-FU exposure correlated with no response to 5-FU in colorectal cancer patients.[42] Evidence has accumulated that insufficent inhibition of TS by FdUMP may be a major resistance mechanism.[43]

The second mechanism suggested to underly 5-FU resistance is related to the activity of folylpolyglutamate synthase (FPGS), the enzyme responsible for folate polyglutamation. As described above, LV acts as a precursor for CH_2THF, which supports the formation of the ternary complex between TS, CH_2THF and FdUMP resulting in irreversible TS inhibition. Reduced folates inside the cells are present as polyglutamated forms of CH_2THF and exhibit an increased affinity for TS as compared to the non-polyglutamated form. Furthermore, polyglutamation increases the intracellular retention of reduced folates. Thus, polyglutamates of CH_2THF achieve an even better inhibition than CH_2THF itself.[44] A decreased activity of FPGS has been associated with resistance to 5-FU.[45] The determination of FPGS activity may therefore be a promising tool for identifying 5-FU/LV-responsive tumors.

A third mechanism possibly involved in 5-FU resistance is the enzyme dihydropyrimidine dehydrogenase (DPD). DPD activity is high in normal liver and variable in tumors.[46] DPD is the first and rate-limiting enzyme of 5-FU catabolism.[47] Since a high rate of 5-FU catabolism may be one of the reasons of 5-FU resistance and high DPD activity results in a high rate of catabolism, DPD activity in tumor cells is a factor significantly related to 5-FU sensitivity.[48] Intratumoral DPD of complete responders has been compared with partial or non-responders in head and neck cancer patients treated with 5-FU and a trend towards significantly lower activity of tumoral DPD in the complete responders was found.[47]

Clinical results

Biochemical modulation of 5-FU bolus infusion

The most extensive clinical data have been obtained for three modulators, namely LV, MTX and IFN-α. PALA has been investigated to a lesser extent. TMTX is a new and promising modulator in preclinical and early clinical studies. The combination of modulators is still experimental.

In the following paragraphs we will discuss clinical studies in metastatic colorectal cancer with the above mentioned modulators. Combination therapies of 5-FU with a modulator are compared to therapy with 5-FU alone.

5-FU and LV. The results of phase II trials suggest that the combination of 5-FU and LV enhances the activity of 5-FU in patients with advanced colorectal cancer.[49] A substantial number of large randomized phase II and III studies shows improved response rates for 5-FU when dosed in combination with LV.[21]

In 1992 a meta-analysis on nine randomized clinical trials showed a significant benefit of 5-FU plus LV over 5-FU alone in terms of response rate (23 versus 11%, respectively). However, no significant improvement of overall survival was found (11.5 versus 11.0 months, respectively).[50]

High-dose LV did not appear to be superior to low-dose LV.[50-52]

Although weekly and monthly schedules differ in toxicity, with more diarrhea for the weekly regimen and more mucositis for the monthly regimen, no difference in response rate and survival was shown between these schedules.[52]

A caveat in most randomized studies is that 5-FU has been given at equal doses both with and without modulation, resulting in a suboptimal 5-FU dose in the control arm, which was also suggested in the meta-analysis. When 5-FU/LV was compared with equitoxic doses of 5-FU, no difference in treatment outcome was found.[53] The mean 5-FU dose was significantly higher in the 5-FU group (472 versus 420 mg/m^2/week, p=0.0003).

5-FU and MTX. The results of phase II trials suggested an increased response rate on account of MTX modulation from 10-20 to approximately 30%.[54] Subsequent phase III trials yielded equivocal results.[12,21] A meta-analysis showed that MTX has a small but significant advantage over 5-FU alone in terms of response rate (19 versus 10%, $p < 10^{-4}$) and survival (10.7 versus 9.1 months; $p < 0.024$).[55]

The dose of MTX, and the interval between MTX and 5-FU administration have been mentioned as relevant factors.[21] However, in the meta-analysis these factors did not appear to have an impact.[55]

There is a possibility that LV, given as rescue after high-dose MTX, may also act as a biomodulator and may therefore obscure the modulating role of MTX in these schedules.

After 1992 5-FU/MTX has mainly been compared to 5-FU/LV and not to 5-FU alone. Several authors found no difference in therapeutic activity between these treatment combinations and in one study 5-FU/MTX was more toxic.[56-58] In one study an enhanced response rate was demonstrated for 5-FU/MTX with increased toxicity compared to 5-FU/LV given sub-optimal.[59]

5-FU and IFN-α. Based on promising results of the combination of 5-FU and IFN-α in phase II studies, this combination was tested in randomized trials.[60] In these phase III studies no beneficial effect of 5-FU/IFN-α as compared to 5-FU alone or to 5-FU plus LV was demonstrated.[61] Since the combination of 5-FU with IFN-α is associated with a higher toxicity compared to other 5-FU regimens, currently there appears to be no role for this combination.

5-FU and PALA. Many clinical trials of 5-FU and PALA have shown disappointing results. This is probably due to inefficient doses of 5-FU and PALA.[3,12,62] At high doses of PALA, toxic effects become unacceptable. Phase II trials with low doses of PALA administered 24 h before the maximal tolerated dose of 5-FU yielded a response rate of 43%.[23,63]

5-FU and TMTX. Since TMTX does not compete with LV and reduced folates for uptake and polyglutamation, synergistic modulation of 5-FU cytotoxicity may be achieved with the combination TMTX/5-FU/LV. A recent phase II trial of TMTX, LV and 5-FU revealed a 20% partial response rate in 35 previously treated patients[64] and a 50% overall response rate in 30 previously untreated patients with colorectal cancer.[65] Based on these results two international randomized trials comparing 5-FU modulated by LV with or without TMTX have started in 1996 in Europe and in the USA.

Double modulation of 5-FU

On theorectical grounds it may be interesting to combine 5-FU with more than one modulator. With this approach the metabolism of 5-FU may be influenced at different points. With the different mechanism of action of the above-mentioned modulators in mind (Figure 1), one could think of the use of several combinations, e.g. one modulator enhancing TS inhibition and the other increasing incorporation of FUTP into RNA, or two modulators enhancing TS inhibition at a different point.

5-FU and MTX with or without PALA. In the study of 5-FU and MTX with or without PALA, double modulation was not effective.[66]

5-FU and PALA and LV. In another phase II study 5-FU was combined with PALA and LV, and yielded a response rate of only 6.1%.[67]

5-FU and MTX and LV. As already mentioned, LV has to be given 24 h after high-dose MTX as a rescue to protect the normal tissues for toxicity. This time interval of 24 h is important. Increased response rates with no difference in median survival and enhanced toxicity have been observed with this double modulation.[58,59]

5-FU and LV and IFN-α. The addition of IFN-α to 5-FU and LV did not improve response rates, but was found to be more toxic.[68]

Other combinations. Several other combinations of modulators with 5-FU have been applied. 5-FU modulated by MTX and IFN-α had a low response rate and resulted in severe toxicity.[69] Furthermore, other modulators have been added to modulation with LV, MTX or IFN-α. For example, 5-FU has been applied with LV and hydroxyurea and with LV and cisplatin.[70,71] Both combinations were well tolerated in these studies. High response rates were achieved without an apparent survival advantage.

Based on preliminary data the double modulation of 5-FU with TMTX and LV may be more promising.[64,65]

Continuous 5-FU infusion

Flat rate infusion. Commonly, infusional 5-FU is administered at a flat rate.[36]

Chronomodulated infusion. One of the largest studies of chronomodulated chemotherapy was carried out by Levi *et al.* in colorectal cancer patients.[72] They compared chronotherapy with oxaliplatin, 5-FU and LV with constant-rate infusion of these agents. Chrono-therapy was more effective and less toxic than constant-rate infusion.

Biochemical modulation of infusional 5-FU. Effective biochemical modulation of infusional 5-FU has been demonstrated in only one randomized trial. In this trial the effect of MTX modulation was examined and a significant increase of the response rate from 10 to 21% (p=0.025) was shown for modulated infusional 5-FU.[73] However, survival was not improved. Given the low toxicity which was shown in this trial, one could agree that 5-FU doses were suboptimal. The addition of PALA to this 5-FU/MTX regimen in a subsequent randomized trial was not shown beneficial.[66] In several phase II trials no apparent benefit was shown for the biochemical modulation of infusional 5-FU.[74-76]

Discussion

To date, 5-FU remains the backbone of therapy for advanced colorectal cancer. It is important to understand the mechanism of action of 5-FU in order to be able to influence treatment with this drug. To obtain optimal results with biochemical modulation, it is necessary to understand the biochemical background of the different modulators and their interaction with the metabolism of 5-FU. In order to improve the results of treatment, new modulators are under investigation in the preclinical and in the clinical setting. ^{19}F NMR spectroscopy is a method to study 5-FU metabolism in a preclinical as well as in a clinical setting. Factors that are important for 5-FU sensitivity and resistance are the intracellular concentration of reduced folates, the extent of TS inhibition, and the activity of FPGS and DPD. Pre-treatment TS values can be of predictive value for therapy outcome.

Using a variety of modulators, response rates of 5-FU regimens vary between 15 and 30%. The only modulators that have shown efficacy in terms of response rate in randomized trials are LV and MTX. Despite this, the preferred regimen of 5-FU is still a matter of debate.

In spite of a theoretical advantage, to date the combination of several biochemical modulators has not been proven advantageous. In this respect the combination of TMTX with LV may be promising, but the result of randomized trials should be awaited.

With continuous infusion schedules higher dose intensities are achieved. Compared to bolus infusions, this has translated in higher response rates, a more favourable toxicity pattern and a small benefit in overall survival.[36] Possible drawbacks are the need for portable infusion pumps and implanted venous catheters. Data on quality of life and health-care economics for randomized trials are lacking.

Chronotherapy appears to be a way by which a further increase in dose intensity may be reached. To date, however, this approach has also not shown a survival benefit.[72]

There are several new chemotherapeutic agents nowadays which are evaluated for the treatment of colorectal cancer. These include direct TS inhibitors (ralitrex), topoisomerase inhibitors (irinotecan or CPT-11) and a cisplatin analog, showing activity in colorectal cancer (oxaliplatin).[77-79] Furthermore, oral 5-FU prodrugs such as capecitabine (doxifluridine or 5-dFUR) and UFT (ftorafur plus uracil) have promising activity and toxicity patterns.[80,81] In addition, ethynyluracil, an oral dihydropyrymidine dehydrogenase inhibitor, appeared to increase the plasma level of 5-FU and is in early clinical development.[82]

In summary it can be concluded that improved understanding of the biochemical pharmacology of 5-FU has led to the development of several biochemical modulation strategies which are currently being tested in clinical trials. A close collaboration between preclinical and clinical investigation is important in order to get the best results. Until now, 5-FU without modulator given at the maximal tolerated dose has never been proven to be inferior to 5-FU plus modulator. In future studies it is important to use optimal and equitoxic doses of 5-FU, which was often not the case in the past. Only then can the efficacy of new modulators or infusion methods be compared to the currently used treatment.

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