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Review

Comparison of 5-Fluoro-2′-deoxyuridine with 5-Fluorouracil and their Role in the Treatment of Colorectal Cancer

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Despite more than 30 years of intensive studies on new drugs against advanced colorectal cancer, the fluoropyrimidines remain the drugs of choice for systemic treatment and for hepatic artery infusion (HAI). This overview describes new developments in advanced colorectal cancer chemotherapy, providing a rationale for more effective use of the fluoropyrimidines, with biochemical modulation, scheduling or by revealing biochemical mechanisms of action that correlate with antitumour activity. In human colorectal cancer cell lines and various animal tumour model systems 5-fluoro-2′-deoxyuridine (FdUrd) is more effective than 5-fluorouracil (5-FU). Comparably, FdUrd's modulation by leucovorin (LV) is more potent than 5-FU. In animal studies it is shown that intermittent high-bolus administration of FdUrd generates better antitumour activity, compared with equal toxic doses or any other schedule of 5-FU. These effects are related to prolonged thymidylate synthase (TS) inhibition and the prevention of TS induction, rather than RNA incorporation. Preclinical studies with modulators such as *N*-phosphonacetyl-L-aspartate (PALA), WR-2721, mitomycin C and platinum derivatives provide a rationale for clinical use in the future. The first choice systemic chemotherapy of patients with advanced colorectal cancer remains 5-FU combined with LV. Some improvement in therapeutic efficacy has been achieved with locoregional HAI. In randomised studies HAI FdUrd improves the quality of life and survival as compared with optimal systemic therapy. Chronomodulation decreases toxicity, allowing dose intensification, while modulators such as LV or dexamethasone increase survival of patients treated with HAI FdUrd to 86% after 1 year. In conclusion, the clinical use of FdUrd has not been fully explored. Intermittent high-dose FdUrd, chronomodulation together with the use of modulators or drugs focused on prolonged TS inhibition, should be studied in large randomised studies. © **1998 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

COLORECTAL CANCER is the third leading cause of death from all cancers in both sexes in Europe [1]. Patients can present with liver metastases as the first or only sign (as found in 20% of all deceased patients) of advanced disease [2, 3]. Treatment of metastatic disease frequently involves chemotherapy which can be given systemically or regionally. The fluoropyrimidines, 5-fluorouracil (5-FU) and 5-fluoro-2′-deoxyuridine (FdUrd) [4, 5] (Figure 1) are the first choice for the treatment of patients with advanced colorectal cancer [3, 6–9]. With regional hepatic artery infusion (HAI), liver metastases are almost exclusively perfused. This administration is more effective than portal vein infusion which only supplies hepatocytes. Drugs with high hepatic extraction such as the fluoropyrimidines cause minimal systemic toxicity, allowing the

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administration of higher and more effective locoregional concentrations.

5-FU with its modulator leucovorin (LV) is regarded as standard systemic chemotherapy for patients with advanced colorectal cancer [3, 7, 9–11]. FdUrd is the deoxyribonucleoside derivative of 5-FU. While it partly acts as a prodrug of 5-FU, there are many other subtle chemical differences. Its mechanism of action (Figure 2) is thought to be due to the 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP)-mediated inhibition of thymidylate synthase (TS). 5-FU is cheaper with less systemic toxicity, so usage of FdUrd has been restricted to HAI or infusions using chronotherapy. FdUrd and 5-FU differ in their preclinical antitumour activity, clinical pharmacological characteristics and toxicity patterns [3, 6, 12]. To optimise the potential of FdUrd, chronomodulation and addition of modulators in the HAI setting were tested [3, 13, 14]. This overview compares FdUrd with 5-FU in terms of mechanisms of action, preclinical antitumour activity and application in the clinic. Using appropriate administration schedules, FdUrd showed significantly better antitumour activity in animal models compared with 5-FU. We conclude that recent insights into mechanisms of action of FdUrd suggest that the use of this drug is not optimal in the clinic.

UPTAKE AND METABOLISM OF FdUrd AND 5-FU

Both fluoropyrimidines require cellular uptake and metabolism to active products before exerting cellular effects. Ultimately, four metabolic effects of fluoropyrimidines can be distinguished: (1) inhibition of the key enzyme of *de novo* pyrimidine synthesis, TS, by the formation of FdUMP, and subsequent interference with DNA synthesis, (2) incorporation of 5-fluorouridine-5′-triphosphate (FUTP) into RNA thereby interfering with RNA synthesis, (3) incorporation of 5-fluoro-2′-deoxyuridine-5′-triphosphate (FdUTP) into DNA, and (4) the formation of 5-FU sugar derivatives [15].

The predominant metabolic pathway varies according to the fluoropyrimidine used and is determined by levels of the various intracellular enzymes for anabolism and catabolism together with the availability of competing normal substrates and cofactors. This availability varies according to the drug administration schedule as well as the tissue studied. The key mechanisms causing antiproliferative effects are believed to be TS inhibition and RNA incorporation [16–29]. The antitumour activity of 5-FU is postulated to be mainly through inhibition of TS especially when modulated by leucovorin (LV), whereas side-effects are related to RNA incorporation [17, 23, 29]. Furthermore, the mechanism of action may be a function of the schedule of drug administration; TS inhibition occurs with protracted infusion and RNA incorporation occurs with i.v. bolus. The reversal by uridine and uridine diphosphoglucose (UDPG) of 5-FU-induced myelosuppression [23–26] and gastrointestinal toxicity [26, 27] without tumour protection supports this hypothesis. UDPG [27] administration enabled a 1.5-fold increase in 5-FU dosage; the better therapeutic index was most likely due to a prolonged TS inhibition, since UDPG decreased 5-FU incorporation into tumour RNA [28]. In addition, other murine studies demonstrate gastrointestinal toxicity due to RNA incorporation and not to increasing FdUMP levels [29]. Although 5-FU incorporation into RNA seems to be the major mechanism leading to 5-FU toxicity, inhibition of TS as an additional mechanism cannot be excluded since 5-FU administration can inhibit TS in mucosal tissues of mice and patients [30] and in bone marrow cells of rats and mice [30, 31]. The extent of TS inhibition is dependent on the administration route, with isolated liver perfusion resulting in the lowest TS inhibition [31]. In livers from mice, rats and patients, however, 5-FU did not inhibit TS but induced a 2–3-fold increase in TS levels [30, 31]. We conclude that 5-FU toxicity is predominantly caused by 5-FU incorporation into RNA.

Uptake and clearance

In L-1210 cells [32] and in various human tumour cell lines and erythrocytes [33], FdUrd can be transported by a facilitated diffusion transport system (insensitive to nitrobenzylthioinosine). Rapid and not rate-limiting uptake of 5-FU occurs by facilitated transport [34]. Since negatively charged nucleotides cannot leave the cell, the active phosphorylated

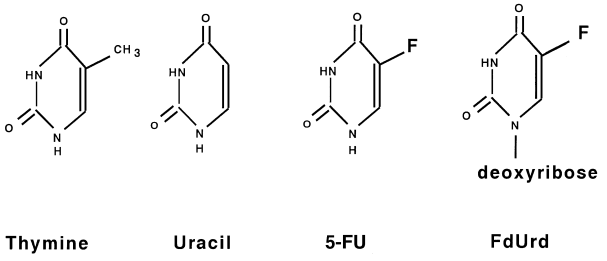

Figure 1. Chemical structures of uracil, thymine, 5-fluorouracil (5-FU) and 5-fluoro-2′-deoxyuridine (FdUrd).

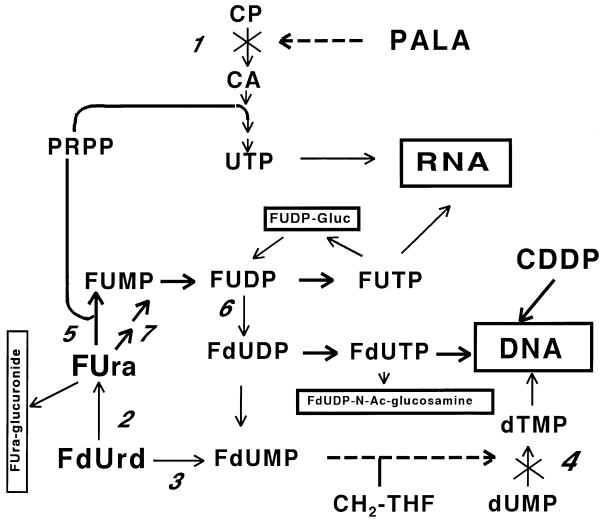

Figure 2. A simplified scheme of the anabolic routes of 5-FU and FdUrd activation and the interference with their modulators, *N*-phosponacetyl-L-aspartate (PALA), cisplatin (CDDP) and leucovorin (metabolised to 5, 10 methylene-tetrahydrofolate, CH_2-THF). CA, carbamyl aspartate; CP, carbamyl phosphate; UTP, uridine-5′-triphosphate; dTMP, 2′-deoxythymidine-5′-monophosphate; dUMP, 2′-deoxyuridine-5′-monophosphate; FUMP, 5-fluorouridine-5′-monophosphate; FdUMP, 5-fluoro-2′-deoxyuridine-5′-monophosphate; FUTP, 5-fluorouridine-5′-triphosphate; FdUTP, 5-fluoro-2′-deoxyuridine-5′-triphosphate; PRPP, 5-phosphoribosyl-1-pyrophosphate. Enzymes: 1, aspartate carbamoyl transferase (inhibited by PALA); 2, thymidine phosphorylase (TP); 3, thymidine kinase (TK); 4, thymidylate synthase (TS) (inhibited by FdUMP); 5, orotate phosphoribosyl transferase (OPRT); 6, ribonucleotide reductase; 7, sequential uridine phosphorylase-uridine kinase. CDDP forms DNA–platinum adducts.

fluoropyrimidine derivatives can accumulate in the cell [35]. In murine colon 26 tumours, rapid and extensive uptake occurs, allowing cellular 5-FU levels to be detected for up to 10 days [36, 37]. The elimination patterns of 5-FU after administration of 5-FU or FdUrd are similar in these studies, but 5-FU is cleared more rapidly from plasma resulting in a high tissue/plasma ratio (> 10) in patients [36].

Anabolism

After uptake, phosphorylation into the active nucleotides takes place (Figure 2). FdUrd can be phosphorylated directly by thymidine kinase (TK) to FdUMP, or cleaved by thymidine phosphorylase (TP) to 5-FU. In some circumstances, but not generally, FdUrd can act as a prodrug of 5-FU [22, 33, 38, 39]. FdUrd possibly has a dual mode of action. FdUMP can be phosphorylated to FdUDP and subsequently to FdUTP by pyrimidine nucleoside mono- and diphosphate kinases.

The predominant activation step for 5-FU is its phosphoribosylation to 5-fluorouridine-5′-monophosphate (FUMP) [40]. This reaction is catalysed by orotate phosphoribosyl transferase (OPRT) requiring the phosphate-donor 5-phosphoribosyl-1-pyrophosphate (PRPP) as a cosubstrate [41]. FUMP can also be formed in a two-step process via FUrd, catalysed by uridine phosphorylase, whose activity is elevated in proliferating tissues [40, 42], followed by uridine kinase. FUMP can be converted into FUDP by pyrimidine nucleoside monophosphate kinase [43]. FdUDP is then formed from FUDP by ribonucleotide reductase and can subsequently be phosphorylated by a non-specific pyrimidine nucleoside diphosphate kinase [44] to FdUTP using ATP as a cosubstrate [44].

TP is a bidirectional enzyme which theoretically can form FdUrd from 5-FU. However, in colorectal tumour tissues, the predominant direction of TP is toward formation of 5-FU from FdUrd due to lack of sufficient levels of the deoxyribose 1-phosphate donor [40, 45]. Direct conversion of FdUrd to 5-FU is suggested in murine studies, where 5-FU after rapid FdUrd injection was present in tumour tissue for a longer period and in a higher concentration than in plasma [12, 37]. These differences between whole body (plasma) and tumour 5-FU kinetics might be explained by tissue-specific isoenzymes of TP in mice and humans [46].

Catabolism

The active metabolites of both fluoropyrimidines can be converted back to 5-FU by several enzymes including 5′-nucleotidases, phosphatases and phosphorylases (including TP) [47–49]. Free 5-FU itself can be degraded by dihydropyrimidine dehydrogenase (DPD), the first enzyme in the catabolic pathway. Reduction by DPD results in dihydrofluorouracil formation, followed by further enzymatic steps to β-ureidoproprionate (by dihydropyrimidinase) and to fluoro-β-alanine, CO_2 and NH_4^+ (by ureidoproprionase). DPD is believed to play a role in sensitivity to 5-FU [50, 55], since congenital deficiency or low activity of DPD has been associated with enhanced toxicity and an increase in plasma 5-FU levels [52–54]. However, in populations of patients without this deficiency, clinical evidence of a relationship between DPD activity and response to treatment with 5-FU and cisplatin (CDDP) has not been found [55]. Thus, the importance of DPD may be limited to a relationship with toxicity, but not directly with antitumour activity.

MECHANISMS OF ACTION

Thymidylate synthase inhibition

FdUMP inhibits TS, the key enzyme in *de novo* pyrimidine synthesis [7, 18, 56, 57]. This inhibition can be augmented by co-administration of LV, which is the precursor of the TS co-substrate 5, 10-methylene-tetrahydrofolate (CH_2-THF). When adequate amounts of CH_2-THF are present, TS, FdUMP and CH_2-THF form a stable ternary complex [58–62], which markedly increases the extent and duration of cellular TS inhibition, thereby enhancing antitumour activity. If CH_2-THF is present in inadequate amounts, FdUMP can dissociate from the enzyme allowing it to function. The kinetics of this process have been well described [59]. In patients, most pronounced inhibition was observed immediately after 5-FU administration (median residual TS activity of 46, 65 and 74% of total TS after 2, 23 and 45 h, respectively); with LV, TS activity was still 49% after 45 h [17, 63], demonstrating the essential role of reduced folates in maintaining enzyme inhibition in patients.

Incorporation into RNA

FUTP acts as a substrate for RNA polymerase and thus will be incorporated into mainly nuclear RNA [20, 64, 65]. Interference with the maturation of nuclear RNA might be related to cytotoxicity [7, 20, 65]. After injection of a therapeutic bolus dose of 5-FU, fluorinated nucleotides have been found in the RNA of tumours in animals for at least 1 week [37] and for 3 days in tumours of patients [66]. No relationship between 5-FU incorporation into RNA and response to 5-FU treatment has been observed in patients [63], while in the same group of 40 patients low TS levels and a high TS inhibition were related to response and high TS levels with no response [17, 63].

Incorporation into DNA

FdUTP acts as a substrate for DNA polymerase and can be incorporated into DNA. However, after 5-FU treatment, DNA incorporation is only 10% of RNA incorporation [67–70] and is not considered an important mechanism of 5-FU cytotoxicity. In contrast, after FdUrd administration to CHO-K1 cells, equal percentages of incorporation into DNA and RNA are achieved with approximately 4-fold more incorporation into DNA after FdUrd than after 5-FU [70]. Furthermore, DNA single- and double-strand breaks occur in several cell lines after fluoropyrimidine treatment and are considered important determinants of FdUrd cytotoxicity but not of 5-FU [68, 71]. Hence, it is feasible that DNA incorporation may contribute to the cytotoxicity of FdUrd, but not of 5-FU. However, the contribution of DNA incorporation to the overall effects of FdUrd has not yet been properly established.

Formation of sugar derivatives of 5-FU

FUDP sugars can be formed after 5-FU treatment [15, 16, 72–75] in various types of cells, serving as alternative substrates for the enzymes of UDP sugar metabolism [47]. FdUDP-*N*-acetylglucosamine and 5-FU glucuronide have been detected in human lymphoid cells [76] and rat hepatocytes [15, 49, 77], respectively. These sugars might serve as enzyme substrates, resulting in prolonged cell exposure to fluorinated nucleotides [15, 47], but may also affect glycosylation reactions in tumour cells [15, 78]. However, formation of sugars is not considered to play a significant role in the cellular effects of fluoropyrimidines.

PRECLINICAL ANTITUMOUR ACTIVITY IN COLORECTAL CANCER

In vitro *studies*

Growth inhibition and modulation of FdUrd and 5-FU by LV. Table 1 summarises the results of five studies [16, 79–82] which compared the two fluoropyrimidines with and without LV in a variety of colorectal cancer cell lines. When given alone, FdUrd was more cytotoxic than 5-FU as expressed by the IC_{50} (molar concentration causing 50% growth inhibition) in a wide range of tumour cell lines. After at least 72 h incubation, the IC_{50}s of 5-FU ranged from 0.8 to 950 μM (data not shown) and FdUrd ranged from 0.36 nM to 3400 μM. In all but three cell lines, FdUrd was more cytotoxic than 5-FU on a molar basis. The dose–effect curve for each drug was sigmoidal. The modulation of FdUrd by LV was more pronounced, with median factors of modulation at their IC_{50}s of 1.5 for 5-FU and 2.6 for FdUrd (Table 1). In most (88%) of these cell lines, FdUrd showed greater potentiation by LV than 5-FU. Furthermore, the cytotoxicity of FdUrd was enhanced by LV (potentiation factor > 1.5) in 17 out of 24 cell lines, compared with only 9 out of 24 cell lines for 5-FU. Although less pronounced, the same phenomenon was observed in a panel of gastric cancer cell lines [80]. In non-small cell lung cancer cells, neither fluoropyrimidine was modulated by LV in these cell lines which were resistant to fluoropyrimidines compared with colon cancer cell lines [80]. The difference between 5-FU and FdUrd may be due to the observation that in cell lines 5-FU can have different mechanisms of effect (RNA and DNA incorporation, or TS inhibition), which may not all be affected by LV. TS inhibition after FdUrd seems to be more general. Mechanistic differences may explain why modulation is different between the various tumour types.

Duration of drug exposure. In accordance with their cell cycle specificity, prolonged exposure of human cell lines to 5-FU or FdUrd greatly enhances growth inhibition [18, 71, 83, 89]. In HCT-8 cells, a 24-fold increase in exposure time to FdUrd from 3 to 72 h led to a 38-fold reduction in IC_{50} [83]. Remarkably, the modulation factor of LV in this study significantly decreased from 3.8 after 3 h to 2.0 after 72 h. In contrast, Moran and associates [79] found that prolonged exposure of WiDR cells with 5-FU led to increased enhancement of its cytotoxicity by LV [79], which was also observed by us in SW948 cells (Van der Wilt and colleagues, University Hospital VU, Amsterdam).

In summary, many *in vitro* studies show that growth inhibition due to FdUrd is greater than 5-FU. The difference is even more pronounced when LV is added, suggesting a significant role for TS in the cytotoxic effects of FdUrd. With a shorter exposure to FdUrd, LV does enhance the formation and stability of a ternary complex, thus prolonging inhibition of the catalytic activity of TS. In contrast, when the duration of FdUrd exposure is prolonged, the influence of LV modulation is decreased [83]. Apparently the binary complex between FdUMP and TS remains relatively stable during prolonged exposure to FdUrd so that addition of LV causes no enhancement of inhibition of TS catalytic activity. For 5-FU, addition of LV may cause a shift in its mechanism of

Table 1. In vitro *cytotoxicity of* 5-*fluorouracil and* 5-*fluoro-2′-deoxyuridine alone and with LV modulation in human colorectal cancer tumour cells*

Cell line	Drug exposure (days)	IC_{50}FdUrd (nM)	IC_{50} ratio*	Potentiation by LV		Reference
				FdUrd†	FUra†	
WiDR	3	4.0	625	8.0	2.5	79
WiDR	9–20	12.5	134	3.1	1.5	80
DLD-1	9–20	129.5	37	3.5	0.8	80
LOVO	9–20	34.4	96	3.9	1.5	80
COLO201	9–20	116	32	5.4	3.1	80
COLO320DM	9–20	80.5	12	4.6	1.4	80
COLO320	6	27.9	344	5.3	1.3	81
SW948	6	0.67	491	2.4	2.3	81
NCI-H548	4	3.4×10^6	0.17	1.1	1.0	82
NCI-H630	4	17×10^4	0.35	1.6	1.3	82
NCI-H684	4	2.6×10^3	13	1.3	1.1	82
NCI-H508	4	1.1	150	1.5	1.5	82
NCI-H747	4	1.3×10^3	11	2.0	1.4	82
NCI-H716	4	4.7	5000	2.0	1.6	82
NCI-H498	4	38.4	143	2.2	2.7	82
SNU-C1	4	0.36	1333	1.7	1.8	82
SNU-C2a	4	12	11	1.6	1.6	82
SNU-C4	4	0.65	2545	2.1	1.7	82
SNU-C5	4	133	113	1.9	1.6	82
WiDR	3	3100	1.6	1.0	1.5	16
WiDR/F	3	2200	2.7	0.7	0.6	16
C26-10 (m)	3	4	133	1.3	0.9	16
C26-10/F (m)	3	15	43	2.5	0.9	16
CC 531 (r)	3	5200	0.35	0.9	1.4	16

In vitro cytotoxicity studies [79–82] in well-established human colorectal cancer cell lines comparing fluoropyrimidines and modulation by LV. *Ratio of the IC_{50} of 5-FU over FdUrd (on a molar basis). †Ratio of the IC_{50}s of each fluoropyrimidine without and with leucovorin (LV). LV was given simultaneously with the fluoropyrimidines at a concentration of 10–20 μM (except by Sinnige [81] who varied LV from 1–100 μM). The mean potentiation ratios for FdUrd and 5-FU are 2.6 ± 1.7 and 1.5 ± 0.6, respectively ($P < 0.05$, Student's *t*-test for unpaired data) which indicate overall greater potentiation of FdUrd by LV than 5-FU by LV.

action from RNA incorporation to TS inhibition, resulting in enhanced growth inhibition in several cell lines. Under conditions of metabolic modulations by LV, drug incorporation into cellular RNA is not altered significantly, neither in cell lines nor in tumour samples from mice [28] and patients [63]. These observations suggest that the cytotoxic effect of 5-FU can be optimised through LV modulation by maintenance of TS inhibition, while cancer cells treated with FdUrd and LV do not require prolonged exposure to achieve high levels of growth inhibition.

In vivo *studies*

In the last two decades many studies comparing 5-FU with FdUrd in various animal tumour models yielded insight into the relative effects of each drug alone, the probable best schedule for each drug in human studies and the level of benefit to be expected from modulators.

Comparison of FdUrd with 5-FU. Early *in vivo* experiments comparing FdUrd with 5-FU were performed by Corbett and associates in 1977 [85]. In various schedules (bolus administrations for 5 days, or weekly for 2–4 weeks) and different colon tumours (Colon 26, -36, -38 and -51), FdUrd yielded better therapeutic efficacy and more tumour-free survivors than 5-FU (6/69 versus 2/90, respectively). The highest responses for both drugs were seen in Colon 38. Studies by our group using various schedules (5 days continuous infusions, 5 days i.v. bolus, 3 weekly one hour infusions or 3 weekly i.v. bolus) in mice with Colon 26-B carcinoma revealed better antitumour activity of FdUrd compared with 5-FU at both equimolar and maximum tolerable doses. The most effective treatment schedule of FdUrd was a weekly i.v. bolus injection for 3 weeks. This schedule dependency is not observed with 5-FU treatment [12]. FdUrd is also more effective than 5-FU in rat models. The most effective schedule was also weekly high-dose administration [83]. Bartowski [86] studied rats bearing Novikoff hepatoma treated with either 5-day continuous infusion or five daily bolus injections. FdUrd had better antitumour activity than 5-FU at equimolar doses. With FdUrd the best antitumour activity was seen after continuous infusions. However, the maximum tolerated dose (MTD) was not determined and a weekly schedule was not studied. Continuous intra-arterial administration was the only route and schedule to produce significant antitumor activity for 5-FU in this study. In a similar experiment, rats bearing subcutaneous Novikoff hepatoma were given FdUrd via the femoral artery [87]. Five out of 6 rats (83%) had responses with five daily bolus administrations compared with 50% with equimolar doses given by continuous infusion.

Human colorectal xenograft tumours ($HxELC_2$ and $HxHC_1$) in immune-deprived mice showed better antitumour activity for one bolus injection of FdUrd compared with an equitoxic 5-FU dose [88]. Murine studies with Colon 26 by Iigo and associates [89] showed similar activity of 5-FU and FdUrd when given once i.p. After weekly therapy, superior antitumour activity of 5-FU compared with FdUrd was seen in the murine Colon 38 tumours [90]. This favourable antitumour activity of 5-FU compared with FdUrd as well as the antitumour activity of 5-FU differ from the results of other groups and might therefore not be representative [85, 91].

These findings show that intermittent FdUrd bolus administration is the best schedule of administration in these tumour models, and is better than any schedule of 5-FU.

Biochemical modulation. Extensive scheduling and dose-finding studies with LV revealed potentiation of the antitumour activity of 5-FU by LV given before and during 5-FU therapy in murine Colon 26-A and Colon 38 [60]. The Colon 38 tumour has lower TS activity, which may account for its greater sensitivity [61]. In both tumours the significant effects of LV on residual TS activity only occurred after 3 weeks of weekly administration [61]. First, inhibition of TS induced by LV plus 5-FU was more pronounced than 5-FU alone; second, LV prevented the 5-FU-induced increase in TS, which is seen in mice treated with 5-FU alone. Similarly, in another (Colon 26-B) model, superior FdUrd antitumour activity compared with 5-FU was related to more prolonged TS inhibition [37]. In another study, measurements of TS limited to 1 day in the Colon 38 tumour model of Iigo and associates [90] were too incomplete to correlate the antitumour activity of fluoropyrimidines with the extent of TS inhibition. In measurements of such short duration, LV is not likely to potentiate an almost complete TS inhibition. Although these *in vivo* data are limited, it might be concluded that prolonged TS inhibition and prevention of TS induction are of significant importance in the antitumour activity of fluoropyrimidines. The data between the various models are very different; when translated to the clinic, various different schedules may be used for different tumours.

Selective enhancement of the biochemical effects of fluoropyrimidines in tumours might improve therapeutic efficacy. Various strategies have been developed and evaluated by our group, using *N*-phosphonacetyl-L-aspartate (PALA) and CDDP as modulators [12, 91–94] in mice bearing Colon 26-A and B tumours. The antitumour activity of FdUrd was enhanced by PALA and even more so with a triple combination of PALA followed one day later by CDDP and FdUrd at an active dose [93]. This combination resulted in 66% complete responses (CR) compared to 25% with FdUrd alone and none with PALA + CDDP [12, 94]. The antitumour activity of 5-FU was enhanced by PALA from 10% partial responses (PR) to almost 60%, of which 10% were complete. Addition of a very low dose of CDDP resulted in 100% remissions of which 30% were complete [94]. Apparently triple PALA/fluoropyrimidine/CDDP combinations might be more effective than only two of these agents. The mechanism of action of PALA in this model appears to be through reduction in pyrimidine nucleotide pools, permitting unopposed fluoropyrimidine nucleotide action. The potentiation by PALA was related to the duration of decreased nucleotides [12]. Inhibition of TS activity by CDDP observed in Colon 26-A bearing mice treated with the chemoprotector WR-2721, 5-FU and modulating doses of CDDP might explain the synergism between fluoropyrimidines and platinum compounds [91].

Locoregional therapy. The aim of regional therapy is to improve the therapeutic index by delivering the highest concentrations of anticancer drugs directly to the site of the tumour achieving less systemic toxicity. Bartowski and associates [86] used rats bearing Novikoff hepatoma and showed that the highest tumour responses were found after administration of FdUrd directly into the hepatic artery compared with administration via the vena cava or portal vein. This finding is consistent with the known blood supply of liver metastases. Riemenschneider and associates [95] compared the effect of continuous infusions to intermittent locoregional therapy with FdUrd in the same animal system. Liver metastases treated by HAI from days 5 to 12 with

420 mg/kg FdUrd showed a low but significant tumour reduction. A bolus administration of 210 mg/kg on days 5 and 8 was ineffective. However, the schedule dependency of FdUrd was not taken into account as equal doses of FdUrd were used; much higher doses of FdUrd could have been used in the bolus arm of the study to achieve equal normal tissue toxicity.

In an attempt to overcome acquired resistance and minimise toxicity, Arisawa and colleagues used HAI FdUrd with or without mitomycin C in rats bearing K12/TRb liver metastases [96]. Treatment and resistance were evaluated by removal of the tumour after 28 days, and preparation of a single cell suspension followed by evaluation with a MTT assay. The antitumour activity of FdUrd with and without mitomycin C was comparable. However, decreased acquired resistance and toxicity were observed with 7 days of HAI FdUrd + one bolus dose of mitomycin C compared with 14 days of HAI FdUrd alone.

Summary of in vivo *studies*

These *in vivo* studies suggest that optimal FdUrd administration is with high-dose bolus injections. Furthermore, most studies show better therapeutic index for FdUrd compared to 5-FU. Also the use of modulators such as PALA, LV and CDDP leads to improvement of the therapeutic efficacy, while co-administration of mitomycin C might diminish acquired FdUrd resistance of hepatic tumours. Chronomodulation of continuous infusions can permit the delivery of a higher daily dose with less overall toxicity, due to circadian variation in TK and DPD [97–99]. The relative value of chronomodulation compared with intermittent bolus is unclear. However, in most circumstances, intermittent bolus injections are technically easier.

CLINICAL TRIALS WITH 5-FU OR FdUrd IN ADVANCED COLORECTAL CARCINOMA

Systemic FdUrd or 5-FU

In the 1960s, various groups compared i.v. FdUrd with 5-FU in patients with advanced colon cancer [99–102] (Table 2). Most studies showed no difference, but Ansfield and associates [99] showed significantly better response rates for FdUrd, possibly because of prolonged duration of treatment. In this study patients were treated for 11 consecutive days every 4 weeks, whereas in the other studies patients had shorter treatment periods [100–102] (Table 2).

Moertel and colleagues [103] compared rapid intravenous injection of FdUrd with continuous intravenous therapy in 128 patients. They concluded that 18-fold greater dose intensity could be achieved with bolus treatment for the same toxicity level. In addition, significantly more objective regressions were found in the rapid injection group (17.5 versus 6.2%, respectively). Unfortunately, confirmatory studies have not been performed and the importance of this trial in considering the optimal administration schedule of FdUrd has been overlooked. Due to lower toxicity and costs, systemic 5-FU therapy has been preferred over FdUrd therapy [47]. Studies of the comparative efficacy and toxicity of FdUrd and 5-FU in large randomised clinical trials using current evaluation criteria are needed.

Hepatic arterial infusion

Ensminger and colleagues [6] showed that FdUrd was almost completely extracted (94–99%) by the liver compared to the 19–51% extraction of 5-FU, which is still much better than many other anticancer drugs [3, 6]. Thus, FdUrd is ideally and 5-FU moderately suited to the pharmacological principle underlying HAI, in that the therapeutic index may be more favourable for those drugs by this route compared with systemic delivery. This hypothesis was tested in only one study in the late 1970s. HAI of 5-FU was compared with systemic administration of 5-FU in 61 patients with liver involvement of colorectal cancer. No significant differences in tumours responding were seen [104].

HAI of FdUrd has been evaluated in many clinical studies of advanced colorectal cancer [105–112], although not all studies were randomised [105]. In general, response rates have been superior to any systemic therapy, but possible survival benefits have been obscured by the cross-over of treatments. The median response rates in 10 studies of HAI of FdUrd was 45%, yielding a median survival of 17 months [3]. Three of these studies are shown in Table 3 [106–108], where HAI of FdUrd was compared with systemic FdUrd therapy in advanced colorectal cancer. In two of these studies, the response rate and survival were better for HAI [106, 107]. FdUrd has demonstrated its superiority over systemic 5-FU therapy in terms of response rates in four studies (Table 3; [109–112]). The observations can, however, be criticised, since in most of these trials cross-over was allowed and groups were too small to allow meaningful statistical evaluation [3, 9, 10, 13, 113, 114]. However, if the cross-over effects of the other trials were taken into account, HAI treatment was significantly better [3].

The most instructive studies are the French [110] and English [111] studies, where no cross-over was allowed, and in both trials HAI FdUrd resulted in significantly better median survival than systemic 5-FU therapy alone. Control groups were only treated according to the physician's preference and the HAI group was also treated with systemic

Table 2. Comparison of systemic 5-FU with FdUrd

Reference	Dose/schedule		No. patients	Responses (%)	
	5-FU†	FdUrd		5-FU	FdUrd
Ansfield [99]	15 × 5→7.5 × 11	30 × 5→15 × 11	198	17	44*
Reitemeier [100]	15 × 5→7.5 × 4	40 × 5→20 × 4	168	12	23
ECGSTC† [102]	15 × 4→7.5 × 4	30 × 4→15 × 4	104	27	4
Young [101]	15 × 4→7.5 × tox‡	30 × 4→15 × tox	86	15	16

Early trials [99–102] comparing systemic 5-fluorouracil (5-FU) with 5-fluoro-2′-deoxyuridine (FdUrd) in patients with advanced colorectal cancer. *Drugs were given in mg/kg/d for 4 or 5 days, followed by half the initial dose every other day. Courses were repeated after 4–6 weeks until progression occurred. †Eastern Cooperative Group in Solid Tumor Chemotherapy. ‡Treatment until toxicity occurred. Significant difference ($P < 0.001$) in response rates between 5-FU and FdUrd treatment was seen in one study [99].

Table 3. Comparison of systemic therapy with hepatic artery infusions of fluoropyrimidines

Reference	No. of patients	Therapy		Partial responses %		Median survival (months)	
		HAI	Syst	HAI	Syst	HAI	Syst
Grage [104]	61	5-FU	5-FU	34	23	15.4	13.5
Chang [106]	64	FdUrd	FdUrd	62	17	17	12
Kemeny [107]	162	FdUrd	FdUrd	52	20	17	12
Hohn [108]	143	FdUrd	FdUrd	42	10	16.7	16.1
Martin [109]	74	FdUrd	5-FU	54	21	12.6	10.5
Rougier [110]	163	FdUrd	5-FU	43	9	15	11*
Allen-Mersh [111]	100	FdUrd	5-FU	50	0	13.5	7.2*
Wagman [112]	41	FdUrd	5-FU	56	0	—	—

Randomised studies [104, 106–112] comparing systemic (Syst) fluoropyrimidines with hepatic artery infusions (HAI) of 5-fluorouracil (5-FU) or 5-fluoro-2′-deoxyuridine (FdUrd) in patients with liver metastases of colorectal cancer. Responses were significantly different in all studies except that of Grage [104]. *Significant ($P < 0.05$) difference in survival or quality of life between systemic and HAI therapy was observed in two studies. In all but those two studies [110, 111], cross-over was allowed.

FdUrd therapy when extrahepatic disease occurred. The English group [111] was the only group to include quality of life assessments, and demonstrated improved quality of life for patients randomised to receive HAI FdUrd or best supportive care.

Since significant first-pass hepatic extraction of 5-FU occurs, a phase II study of HAI of 5-FU was conducted in Amsterdam in 37 patients. The overall response rate was 37.5%, which is in the same range as HAI FdUrd. As seen with HAI of FdUrd, no toxic side-effects were observed and patients responding to HAI 5-FU showed better inhibition of tumour TS than non-responders [17, 63]; no difference was observed for 5-FU incorporation into RNA. 5-FU did not inhibit liver TS, but in contrast induced a significant 2–3-fold increase in TS levels [30]. HAI FdUrd has not yet been compared with current optimal systemic therapy (5-FU + LV) in a randomised controlled trial. However, if the outcome of six randomised trials of systemic 5-FU and LV therapy is compared with HAI FdUrd in four other randomised trials [106–108, 110], HAI of FdUrd seems at least similar and possibly better in terms of objective responses. Furthermore, 1 and 2 year survival rates appear much better for FdUrd (Table 4) which may represent greater efficacy [3].

HAI FdUrd is accompanied by local toxicity, such as sclerosing cholangitis in 5–29% and chemical hepatitis [106–108, 110, 118]. In an attempt to reduce these side-effects, Kemeny and associates [115] compared dexamethasone and FdUrd to FdUrd alone in a randomised controlled trial. A remarkable increase in response rate was unexpectedly observed (71% for FdUrd + dexamethasone versus 40% for FdUrd alone, $P = 0.03$, with a trend towards increased survival; 23 months versus 15 months). In addition there was a significant reduction in local toxicity. The mechanism of this modulation remains unclear, but speculation includes protection of liver and endothelial cells by dexamethasone, resulting in the ability to prolong FdUrd therapy because of reduced toxicity.

Table 4. Comparison of systemic 5-FU + LV with hepatic arterial infusion of FdUrd

Administration	Therapy	No. of patients	Partial responses (%)	Survival (%)	
				1 year	2 year
Systemic	5-FU + LV	966	38	52	19
HAI	FdUrd	532	51	66	30

Randomised trials (total of 6 derived from [3]) using systemic 5-fluorouracil (5-FU) + leucovorin compared with four randomised studies [106–108, 110] using hepatic artery infusions (HAI) with 5-fluoro-2′-deoxyuridine (FdUrd) in patients with liver metastases of colorectal cancer, deduced from a review by Kemeny [3].

Biochemical modulation of FdUrd may be beneficial, similarly to 5-FU modulation. A phase II study by Levin and Gordon [116], using a schedule of 6 days infusional LV and bolus FdUrd on days 2–6, showed a high response rate (55%) and high 1- and 2-year survival (73 and 50%, respectively) in previously untreated patients with advanced colorectal cancer, with a low incidence of severe toxicity. Modulation of FdUrd by LV using HAI in hepatic disease produced a median survival of 28.8 months [117]. In this study, high 1, 2, 3, 4 and even 5-year survival rates of 86, 62, 31, 15 and 7% were seen [117]. Dexamethasone was added to this schedule in a phase II study, producing similar survival rates and lower toxicity [118]. Other attempts to increase the therapeutic efficacy include alternating schedules with HAI FdUrd and systemic 5-FU, with beneficial therapeutic efficacy (minimal toxicity and similar survival rates of > 22 months) [119, 120]. These studies suggest that biochemical modulation of FdUrd by LV may be superior to FdUrd alone or to 5-FU + LV, but the strategy needs to be tested in randomised controlled comparative trials. The explanations for additional efficacy of dexamethasone with FdUrd but not with FdUrd + LV is unclear.

An argument against HAI FdUrd is the initial cost (financial costs, as well as the necessity for surgery for catheter and pump or port insertion). However, the overall expenses of HAI therapy are comparable to systemic 5-FU and LV administration after 1 year of treatment [3]. Since the median survival of patients treated with HAI exceeds this limit, HAI therapy cannot be dismissed on the basis of greater expense and the data strongly suggest that it provides better outcomes for patients with liver metastases.

Alternative approaches

Therapeutic 'synergism' between anticancer drugs has been considered worthwhile, whereby a mechanistic interaction may allow greater cytotoxicity in tumour tissues than the sum of the effects of each drug alone, but without greater toxicity in host tissue. In this regard, fluoropyrimidines have been combined with other anticancer agents. Patt and

colleagues [121] used HAI FdUrd + CDDP in patients with liver metastases from colorectal cancer, resulting in 52% response rates in 29 patients. In attempting to optimise total drug administration by giving high-dose i.v. bolus FdUrd with LV, an MTD of 1.65 g/m^2 FdUrd was achieved with 500 mg/m^2 LV in 2 h [22]. Alternatively, FdUrd may be given in the peritoneal cavity which might act as a drug reservoir and achieve prolonged low plasma concentrations reducing the toxicity. Doses of 3 g for three consecutive days could be achieved without toxicity [122]. A number of promising new drugs (e.g. campothecins and specific TS inhibitors) are currently under investigation [123, 124]. A recent randomised study of Tomudex compared to 5-FU-LV (low-dose LV daily × 5 schedule) revealed a 20% response rate for Tomudex compared to 13% for 5-FU-LV therapy [125]. However, the responses for FUra-LV are lower than in the original Mayo Clinic study (43%) [126]. CPT-11 data from randomised studies are awaited. More randomised controlled trials will be needed to demonstrate the benefit of such alternative approaches for patients with colorectal cancer.

Chronomodulation allows higher doses of FdUrd with decreased toxicity [14]. Chronotherapy of HAI FdUrd results in a 30% decrease in toxicity compared with flat-rate infusions [127], but is not yet accepted as a viable alternative to flat-rate infusions or bolus therapy and needs to be studied in large controlled and randomised trials.

CONCLUSIONS

Although much effort has been undertaken to optimise fluoropyrimidine therapy with either 5-FU or FdUrd for patients with advanced colorectal cancer, metastatic disease is still incurable. Although advances have been made, up to now the current treatment of these patients remains palliative. The most important advances, concerning improvement of quality of life and increased survival, are seen when metastases are limited to the liver and locoregional therapy can be administered using hepatic arterial infusions of FdUrd. If extrahepatic disease occurs, locoregional treatment can be combined with systemic 5-FU administration. Promising results are seen when FdUrd is modulated with either dexamethasone or LV and optimisation of the schedule might be reached with chronomodulated therapy.

The use of FdUrd in the treatment of patients with colorectal cancer might be more important than so far has been appreciated. The most effective schedule has yet to be clearly determined. Preclinical studies show different antitumour effects of 5-FU and FdUrd in various tumour model systems and schedules of administration, suggesting that different mechanisms of action may predominate in different circumstances for both fluoropyrimidines. In addition, recent research into the cellular pharmacology of FdUrd and the clinical observations that patients with metastatic colorectal cancer might benefit only from intermittent high bolus dose FdUrd rather than continuous infusions suggest that the optimal clinical administration of this drug may not be by continuous infusion, as had been thought previously. The favourable antitumour activity for systemic bolus FdUrd compared with 5-FU in the murine Colon 26 tumour model system [12], higher *in vitro* activity of FdUrd than 5-FU and reduced acquired resistance when treated for a shorter period with combination therapy [96] suggest that these schedules should be more intensively studied in the clinic. Modulating agents such as mitomycin C, platinum compounds, PALA or LV might be added to improve the outcome of colorectal cancer chemotherapy. When hepatic and extrahepatic disease co-exist, HAI FdUrd combined with systemic 5-FU therapy might improve survival. CPT-11 and Tomudex may not only be important new alternatives for fluoropyrimidine therapy [123–125], but should be evaluated in combination with fluoropyrimidines. Further clinical studies of the utility of fluoropyrimidines are warranted, and may ultimately show that in a specific schedule with or without biochemical modulators, FdUrd has superior efficacy to 5-FU in the management of patients with advanced colorectal cancer. Moreover, optimisation of fluoropyrimidine therapy may prove just as valuable as any of the promising new agents.

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