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Short Communication

Modulation of 5-Fluorouracil with Methotrexate and Low-dose *N*-(phosphonacetyl)-L-aspartate in Patients with Advanced Colorectal Cancer. Results of a Phase II Study

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Methotrexate (MTX) and *N*-phosphonacetyl-L-aspartate acid (PALA) have been shown to modulate the cytotoxic effects of 5-fluorouracil (5-FU). A phase II study was initiated to evaluate the feasibility, toxicity and efficacy of PALA/MTX and 5-FU in patients with metastatic colorectal cancer. 26 patients received PALA 250 mg/m^2 as an intravenous 15-min infusion plus MTX 200 mg/m^2 as a 30-min intravenous (i.v.) infusion on day 1 and 5-FU 600 mg/m^2 as i.v. push on day 2. Cycles were repeated every 14 days and the 5-FU dose was escalated in the individual patient in steps of 100 mg/m^2 for the third, fifth and seventh cycle in the absence of toxicity. 7 patients had received prior 5-FU-based chemotherapy while 19 patients were chemotherapy naive. Objective responses occurred in 23% of patients (1 CR, 5 PR of which 2 were pretreated), no change in 13 patients (50%) and tumour progression (6 patients) or toxic death (one patient) in 27%. Responses lasted for a median of 7 months (range 6–9), the median time to progression was 4 months and median survival 13 months. Toxicity was mainly gastrointestinal with diarrhoea and mucositis, and severe or life threatening in only 3 patients. In 3 patients an increase in serum glucose levels occurred while being treated with PALA/MTX and 5-FU. 2 patients with insulin-dependent diabetes had a 33% increase in insulin requirement and 1 patient with dietary-controlled diabetes died due to a ketoacidotic coma. PALA/MTX/5-FU in this dose and schedule is active in patients with colorectal cancer. Hyperglycaemia may be a potential side-effect of PALA-containing regimens especially in patients with diabetes. Careful monitoring of serum glucose levels in these patients is indicated. © **1997 Elsevier Science Ltd.**

Key words: colorectal neoplasms, PALA, 5-fluorouracil, methotrexate, hyperglycaemia
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INTRODUCTION

THE TREATMENT of patients who have advanced colorectal cancer with biochemical modulations of 5-fluorouracil (5-FU) by folinic acid (FA) or methotrexate (MTX) results in a higher response rate compared with the administration of 5-FU alone [1]. However, the influence on median survival is limited according to the findings of two recent meta-analyses [2, 3], indicating only a 4-month increase for those patients treated with a MTX/5-FU protocol. The development of new treatment options is therefore warranted.

MTX is able to modulate 5-FU effects on RNA and DNA synthesis as a potent inhibitor of the *de novo* pyrimidine and purine synthesis [4, 5]. The blocking of these pathways

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results in an intracellular accumulation of phosphoribosylpyrophosphate (PRPP), a co-factor that is essential for the conversion of 5-FU to fluorouracil triphosphate (FUTP) [6]. FUTP may be incorporated into cellular RNA leading to impairments of RNA processing and functioning with ultimate cell death.

N-Phosphonacetyl-L-aspartate acid (PALA) is a potent inhibitor of aspartate transcarbamylase (ACTase), the second enzyme involved in *de novo* pyrimidine biosynthesis [7]. The inhibition of this enzyme can decrease cellular uridine and cytidine pools. The natural nucleoside uridine and its higher phosphates may compete with 5-FU for metabolising and target enzymes. The depletion of uridine triphosphate pools by PALA may result in increased FUMP and FUTP favouring the incorporation of FUTP into RNA. The decreased formation of dUMP results in less competition with FdUMP for thymidylate synthase (TS) binding.

Preclinical studies have demonstrated that low doses of PALA are biochemically effective and may have a selective effect on the tumour compared with normal tissue [8, 9]. Thus, a single dose of 250 mg/m^2 reduced total body pyrimidine synthesis, this effect being maximal at 24–48 h and lasting for 5 days [10]. PALA should therefore be given prior to 5-FU to allow for a depletion of nucleoside pools. Likewise, preclinical and clinical data have demonstrated that the ability of MTX to modulate 5-FU is also schedule dependent. Patients treated with a 24 h interval between MTX and 5-FU had twice the chance of response (14.5% versus 29%) to therapy compared with patients in which 5-FU was given 1 h after MTX administration, and also had a significantly prolonged survival (11.4 versus 15.3 months) [11]. Sequential administration of MTX and 5-FU appeared suitable for studying the double modulation of 5-FU with PALA and MTX to take advantage of a possible synergistic effect.

PATIENTS AND METHODS

From January 1992 to December 1992, 26 patients with histologically confirmed metastatic colorectal cancer and measurable tumour lesions were entered into the study. All patients had documented disease progression of 25% within a period of 3 months, or deterioration of tumour-related symptoms prior to therapy. All patients were required to have a performance status of ECOG 0 or 1, normal bone marrow (WBC > 3000/μl and platelets > 100 000/μl), renal (serum creatinine < 115 μmol/l and creatinine clearance > 70 ml/min), and liver (bilirubin and transaminases < 2× upper limit of normal) function. Patients were previously untreated or had received not more than one prior chemotherapy regimen for metastatic disease. Patients with ascites or pleural effusions were excluded. All patients gave their written informed consent after the purpose and the potential risks of the study were explained to them. The study had been approved by the local ethics committee and was conducted under the local legal requirements.

PALA was given at a dose of 250 mg/m^2 as a 15-min intravenous (i.v.) infusion on day 1 followed by methotrexate 200 mg/m^2 as a 30-min i.v. infusion on the same day. Patients then received hydration with 1500 ml 5% dextrose or normal saline over 2 h. 5-FU at a dose of 600 mg/m^2 was administered as an i.v. bolus 24 h later (day 2). Folinic acid was given at 15 mg/m^2 orally every 6 h for a total of 8 doses starting 24 h after the administration of MTX. If the patient had nausea and vomiting and was unable to swallow folinic acid tablets, the patient was to be admitted to the hospital to receive 15 mg/m^2 folinic acid i.v. every 3 h for a total of 16 doses.

Serum creatinine and MTX plasma levels were obtained 24 h after MTX administration. If the MTX level at this time was more than 5×10^{-7} mol/l or the creatinine increased > 50%, the folinic acid rescue was to be extended to a total of 12 doses. If the MTX level was greater than 10^{-6} mol/l, the folinic acid dose was to be increased to 25 mg/m^2 every 6 h until the MTX level was below 10^{-7} mol/l. The treatment was repeated every 14 days which was defined as one cycle. For the third, fifth or seventh cycle the dose of 5-FU was escalated individually in steps of 100 mg/m^2 in the absence of myelosuppression (WBC > 2000/μl or platelets > 75 000/μl) and mucositis or diarrhoea of greater or equal to Grade 2 (WHO). In case of myelosuppression or gastrointestinal toxicity on the day of therapy, 5-FU and MTX doses were modified as previously described [11]. In the event of stable disease, 12 courses (i.e. 24 weeks of therapy) were administered if the toxicity was tolerable for the patient. In case of partial or complete response, the therapy was given for two courses beyond maximal response and then stopped. Pretreatment evaluation consisted of clinical history and physical examination, routine laboratory tests, including blood count, serum creatinine, electrolytes and liver function tests. For tumour measurement, chest X-rays, CT scans and ultrasound examination were performed. Prior to the next treatment, a clinical history, physical examination and tumour measurements by the appropriate X-rays and scans were repeated every 4 weeks. Tumour response was defined according to standard WHO criteria.

RESULTS

26 patients with metastatic colorectal cancer and disease progression prior to therapy entered this trial. The patient characteristics are summarised in Table 1. 7 patients had received prior 5-FU/folinic acid therapy, but had tumour progression within 6 months after their first-line treatment was discontinued. The best response to 5-FU/folinic acid was stable disease in these patients. The remaining 19 patients were chemotherapy-naive. The median age was 56 years and

Table 1. *Patient characteristics*

Number of patients	26
Age (years)	
Median	56
Range	32–71
Male	17
Female	9
Pretreated	7
Site of primary	
Colon	19
Rectum	7
Metastasis	
Liver	21
Lung	19
Nodes	7
Skin	1
Bone	1
Metastatic sites	
1	7
2	11
> 2	8

the median performance status was ECOG 0. Most patients (81%) had liver metastasis and 7 patients had only one metastatic site.

Drug administration and toxic effects

A median of 6 cycles (range 2–14) was administered. 5-FU was escalated in 8 patients up to 700 mg/m^2 (31%), in 6 patients to 800 mg/m^2 (23%) and in 5 patients to 900 mg/m^2 (19%). 7 patients received 600 mg/m^2 (27%). The percentage of patients with 5-FU dose escalation was 73%. The mean 5-FU dose intensity was 304 mg/m^2 per week (± 63 mg/m^2 S.D.) with a median of 300 mg/m^2. The toxicity pattern is demonstrated in Table 2. Mucositis, diarrhoea and nausea were the dominant toxic effects, being severe and life threatening in 3 patients. The haematological side-effects were mild. No case of nephrotoxicity or neurotoxicity was observed. A 61-year-old female died due to a ketoacedotic coma that developed after she had received the second cycle of chemotherapy. She was a known dietary controlled diabetic with stable glucose levels for years. The patient had not received any medication known to alter serum glucose levels and was otherwise well without any other concomitant disease. 2 male patients with insulin-dependent diabetes noticed an approximately 33% increase in insulin requirement during the 3 days following MTX and PALA administration. The increased serum glucose levels in these patients were documented by regular self-measurements and occurred after every cycle of chemotherapy. None of these patients received intravenous glucose infusion after MTX administration.

Response and survival

One complete response and five partial responses were observed occurring in liver, lung and lymph node metastases. 2 of the responding patients had received prior chemotherapy and 4 patients were chemotherapy naive (1 CR and 3 PRs). The responses occurred after the fourth to the twelfth cycle and lasted for a median of 7 months (range 6–9 months). The overall response rate for all 26 patients was 23% (7–40%, 95% confidence interval). No change was observed in 13 patients (50%) and rapid tumour progression within the first 4 weeks was seen in 6 patients (the last patient died after the second cycle, as mentioned above). Responses occurred in patients receiving an escalated 5-FU dose of up to 900 mg/m^2 (1 CR), 800 mg/m^2 (1 PR) and 700 mg/m^2 (4 PRs). No response was observed in patients receiving 600 mg/m^2. The median time to progression was 4 months and the median survival 13 months (Figure 1).

DISCUSSION

Data from randomised trials indicate that the response rate of 5-FU given as single-agent bolus application is well below 20% [1]. Several attempts have been made to increase the antineoplastic efficacy of 5-FU and biochemical modulation had appeared to be successful [2, 3]. The addition of MTX or folinic acid to 5-FU bolus schedules may double the rate of objective responses. Investigators have therefore been attracted to further explore the strategy of biochemical modulation that was always guided by a sound rationale, impressive preclinical data and promising early clinical observations. Potential agents for 5-FU modulation are PALA, interferons, dipyridamole, thymidine, uridine, azidothymidine (AZT), and others that have been studied in various combinations and schedules [12]. Currently, there is no convincing evidence that any of these compounds is able to increase the antineoplastic activity of 5-FU when used in patients.

Table 2. Maximum toxicity (WHO) observed over all cycles per patient

WHO grade	1	2	3	4
Diarrhoea	3	4	2	1
Mucositis	5	7	1	—
Nausea	10	7	2	—
Vomiting	1	1	1	—
Leucopenia	2	—	—	—
Anaemia	2	—	—	—

Nevertheless, the principle of biochemical modulation has been proven to be effective at least for folinic acid or MTX, and it appears attractive to combine modulating agents to take advantage of a possible additive or synergistic effect, often referred to as double modulation. The results of our study demonstrate the feasibility of combining MTX and PALA with 5-FU. A rate of < 10% of grade 3 or 4 (WHO) gastrointestinal side-effects observed in our trial appears to be lower compared with the experience of Marsh and associates with an observed rate of 18% [11]. Individual 5-FU dose escalation might have been possible in more patients and probably at an earlier stage than was allowed in our protocol. We administered increased 5-FU doses only after every second cycle in contrast to the study by Marsh and associates [11], in which 5-FU doses were escalated after each course. Nevertheless, the percentage of patients with 5-FU dose escalation and the median delivered 5-FU dose intensity were equivalent in both trials. A different patient population, recruited only in well-experienced university centres compared with the multicentre study might explain the difference.

Phase I data indicated that 5-FU at a dose of 800 mg/m^2 may be given every other week in combination with PALA and MTX using the same dose and schedule as in our trial [13]. For further investigation of such a schedule a higher 5-FU dose may be feasible and probably indicated to achieve maximum antineoplastic efficacy.

To our knowledge, alterations in serum glucose levels in patients receiving a PALA-containing chemotherapy have not been described before. A measurable and reproducible increase with a definite correlation to chemotherapy was only observed in known diabetics. A syndrome of ascites, hyperbilirubinaemia and hypoalbuminaemia has been described in patients receiving 5-FU plus PALA [14]. Such a syndrome

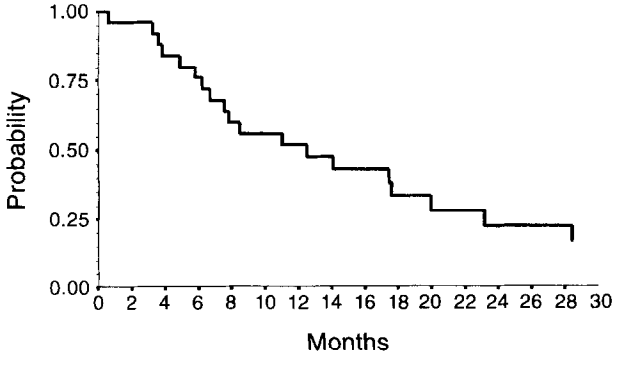

Figure 1. Probability of survival in 26 patients after initiation of therapy.

was not seen in our patients and was also not observed in the 3 individuals with elevations of serum glucose levels. None of these patients had other causes that would explain the altered glucose levels, such as concomitant medication or another concomitant disease. The cause of this unique toxicity remains unclear. It has been speculated [14] that PALA might induce a similar toxicity pattern as L-asparaginase, an enzyme that catalyses the hydrolysis of the non-essential amino acid L-asparaginine to aspartic acid and ammonia. L-Asparaginase is predominantly used in acute lymphoblastic leukaemia, and hyperglycaemia with ketoacidotic coma is a known complication for L-asparaginase treatment as a result of decreased protein synthesis and impaired insulin production [15, 16]. Because PALA is a potent inhibitor of pyrimidine *de novo* synthesis, uridine pools are sharply decreased. One might speculate that a depletion in uridine triphosphate (UTP) could interfere with glycogen synthesis as UTP is required in the following reaction catalysed by the enzyme UDP-glucose-phosphorylase [17]:

$$\text{glucose-1-phosphate} + \text{UTP} \rightarrow \text{UDP-glucose}$$

Our clinical observation should alert clinicians to closely monitor serum glucose levels in individuals with known diabetes receiving PALA treatment.

The response rate achieved with our schedule of MTX/PALA/5-FU appears to be within the range of what has been reported for the use of MTX/5-FU alone [1]. However, our patients were required to have documented disease progression to be eligible for the study. Such a group of patients might represent a subgroup less likely to respond to chemotherapy.

The ability of low-dose PALA to modulate 5-FU has been investigated in two randomised trials using high-dose infusional 5-FU at a weekly dose of 2600 mg/m^2. Unfortunately, the initial promising results of phase II studies [18] could not be confirmed as neither the rate of objective response nor the median patient survival was altered significantly when low-dose PALA was added to high-dose 5-FU [19, 20]. The results of a recent EORTC trial #40909 comparing infusional 5-FU plus MTX with or without PALA will better define the value of PALA in combination with MTX.

The concept of low-dose PALA in combination with 5-FU-based chemotherapy has recently been questioned. The alteration of white blood cell ATCase activity was studied in patients treated with 5-FU/PALA and the nucleoside transport inhibitor dipyridamole in a phase I investigation [21]. In this study 1000 or 500 mg/m^2 PALA were more effective than 250 mg/m^2 to depress ATCase activity. Due to necessary reductions in 5-FU dose intensity when 1000 mg/m^2 of PALA were combined with 5-FU, a PALA dose of 500 mg/m^2 was recommended for further investigations. Whether this higher dose of PALA is more effective than low-dose PALA remains to be demonstrated.

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