Antitumour Activity, Toxicity and Inhibition of Thymidylate Synthase of Prolonged Administration of 5-Fluorouracil in Mice

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Continuous infusions of 5-fluorouracil (5-FU) are increasingly used in the treatment of cancer. Their optimal use, however, has still to be determined since the availability of suitable animal models is limited. We studied continuous infusions in mice using subcutaneously implanted pellets that release 5-FU over a period of 3 weeks. At the maximum tolerated dose (MTD) (based on the systemic toxicity in healthy animals) we assessed the antitumour activity, haematological toxicity, inhibition of thymidylate synthase (TS) in tumours and the concentration of 5-FU in plasma during the 3-week period. We also studied the addition of leucovorin in different schedules. The dose-limiting toxicity was weight loss, and at the MTD of 10 mg of 5-FU released in 21 days per mouse myelosuppression was tolerable (nadir for leucocytes and thrombocytes was approximately 40% of pretreatment levels). In several independent experiments using the 5-FU-resistant Colon 26 tumour, a good antitumour activity was observed during the first part of the infusion, but thereafter the growth of the tumours resumed; the overall effect of continuous infusions was thus comparable to that of bolus injections. Co-administration of leucovorin did not enhance the therapeutic results; depending on the schedule used, it proved ineffective or only increased toxicity. Similar results were obtained with head and neck squamous cell carcinomas and with the 5-FU-sensitive tumour Colon 38. In Colon 26 tumours the TS activity (FdUMP-binding assay) initially decreased to 20–30% of controls and returned to normal after 11 days. In the catalytic TS assay a slight inhibition was observed for the continuous infusion, followed after 11 days by a marked (4-fold) increase in activity. 5-FU plasma levels varied from 0.1 to 1 µM following a circadian rhythm (with a peak at 6 h after light onset), and were maintained during the entire period. Subcutaneously implanted pellets represent a suitable model to study prolonged administration of 5-FU in mice and to evaluate the effect of modulating agents in laboratory animals before transferring data obtained in vitro to the clinic.

Key words: continuous infusion, 5-fluorouracil, thymidylate synthase

INTRODUCTION

5-FLUOROURACIL (5-FU) has been used for more than 30 years for the treatment of cancer, but even though this is one of the most widely employed drugs in clinical oncology, the most effective schedule has yet to be established [1-5]. A daily bolus for 5 consecutive days, one weekly injection, and prolonged infusions have been employed, but with no clear differences in antitumour activity for the different modalities of administration [6]. The pattern of toxicity, however, varies for the different schedules. The dose-limiting toxicity for bolus injections is myelosuppression, while for continuous infusion gastrointestinal toxicity is prominent; mucositis and diarrhoea can be severe, and at times life-threatening, while the hand-foot syndrome is almost exclusively observed with prolonged infusions [7-9].

The potential use of 5-FU continuous infusion has been known for many years: pharmacokinetic and in vitro data support the concept that a constant exposure is more effective than bolus administration even if plasma concentrations with continuous infusion are lower. When cells in culture are treated for a
prolonged period of time the drug shows more activity than in a 1 h exposure followed by culture in drug-free medium [10-14]. In cell lines the 50% growth inhibitory concentration (IC\textsubscript{50}) of 5-FU for a 1 h exposure is approximately 300 \( \mu \text{M} \), while for 24-72 h exposure it is 2-10 \( \mu \text{M} \). Continuous exposure leads to the constant presence of the active metabolite FdUMP in relatively high concentrations [3] so that the inhibition of thymidylate synthase (TS), the target of FdUMP, is facilitated.

During
5-FU exposure the enzyme was inhibited but its activity recovered when the drug was removed [15, 16]. Previously we obtained convincing evidence that in a murine model for colon carcinoma, Colon 26, the relatively poor inhibition of TS and its retention was associated with the limited antitumour effect of 5-FU but that modulation by leucovorin (LV) enhanced TS inhibition and the antitumour activity [17]. In \textit{vivo} experiments showed that, during prolonged exposure of cells to 5-FU, cytotoxicity was due to inhibition of TS while the effect on RNA retained in tissues was prominent during short-term exposure to high concentrations of the drug [16].

The efficacy of 5-FU during continuous infusion may be influenced by the circadian behaviour of levels of plasma 5-FU [18-20], which are related to the diurnal variations in the levels of the 5-FU-degrading enzyme, dihydropyrimidine dehydrogenase (DPD) [19, 21]. In mice, rats and humans evidence is accumulating that various enzymes involved in 5-FU metabolism, such as DPD, uridine phosphorylase and thymidine kinase have a circadian rhythm [21-24]. Such variations in enzyme activity have not been related to plasma 5-FU concentrations during continuous infusion of 5-FU alone.

Optimisation of continuous infusion of 5-FU requires the availability of suitable animal models. Although continuous exposure of cells to 5-FU \textit{in vitro} is more effective than pulse exposure, \textit{in vitro} models lack the possibility of investigating the pharmacodynamics of bolus and continuous infusion of 5-FU; e.g. the selectivity of both protocols cannot be evaluated. Other factors which cannot be studied \textit{in vitro} are the possible effects of circadian variations and accumulation of the drug in tissues.

Recently, we demonstrated that plasma 5-FU levels do not reflect tissue levels [25]. Prolonged continuous infusions are complex to perform in small animals: a venous access is difficult to maintain even for a relatively short time and other devices for continuous infusions including implantable osmotic pumps are only effective for a few days [26, 27]. Long-term infusions in small animals can best be carried out by means of slow-release forms of 5-FU.

In the experiments described in this paper we have used pellets containing 5-FU that are implanted subcutaneously and release the drug for several weeks. We have characterised the maximum tolerated dose (MTD), plasma levels, toxicity, antitumour activity and effect on the target enzyme of 5-FU given as a continuous administration using subcutaneous pellets. We have also examined the possibility of modulating 5-FU activity with LV given in different doses and schedules.

MATERIALS AND METHODS

\textbf{Chemicals}

5-FU for bolus injections was obtained from ABIC (Netanya, Israel). 5-FU pellets were purchased from Innovative Research of America (Toledo, Ohio, U.S.A.). Leucovorin was provided by the Pharmacy department of the Free University Hospital. 6-\textsuperscript{[3H]}-FdUMP (specific activity 20 Ci/mmol) was purchased from Moravek Biochemical Inc. (Brea, California, U.S.A.) and 5-\textsuperscript{[3H]}-FdUMP (specific activity 10.9 Ci/mmol) from Amersham International (Buckinghamshire, U.K.), di-Tetrahydrofolate (Sigma Chemicals Co., St Louis, Missouri, U.S.A.) was converted into 5, 10-methylenetetrahydrofolate by addition of formaldehyde [12]. All other chemicals were of analytical grade and are commercially available.

\textbf{In vivo studies}

Female, Balb/c, C57/B1 and athymic nude mice (NMRI) of 8-10 weeks were obtained from Harlan/Cpb (Zeist, The Netherlands) and were maintained in the Clinical Animal Laboratory of the Free University. All experiments were performed in accordance with the rules for animal welfare established by this institution. Toxicity studies were carried out in healthy female C57/B1 and Balb/c mice. Parameters used to define the MTD were weight loss not greater than 15\% and/or lethality less than 10\%. Investigations on haematological toxicity were performed as previously described in detail [28-30]. Blood sampling was performed by retro-orbital puncture with heparinised capillaries after slight ether anaesthesia [25].

Antitumour activity was studied on the murine colon carcinomas Colon 26 maintained in female Balb/c mice, Colon 38 maintained in C57/B1 mice and on xenografts of the human squamous cell carcinomas of the head and neck, HNX-14C and HNX-OE, maintained in nude mice. Tumours were transplanted subcutaneously in the thoracic region on each flank of the animal [28-31]. Tumour size was measured sequentially twice weekly using a calliper, and the volume was calculated as length \times width \times height \times 0.5. The evaluation of antitumour activity was based on the following parameters: the ratio of the mean volume of treated tumours and the mean volume of control tumours (T/C); the increase in the life span (ILS) defined as the ratio (\times 100\%) of the median life span (MLS) of treated mice divided by the MLS of controls (MLS is measured starting from the first day of treatment); the growth delay factor (GDF) calculated from the median tumour doubling time (TD) of the tumours of treated mice and control mice according the following formula:

\[
\text{GDF} = (\text{TD}_{\text{treated}} - \text{TD}_{\text{controls}})/\text{TD}_{\text{controls}}
\]

\text{TD}_{\text{controls}} is about 3 days for Colon 26, 5 days for Colon 38, 4 days for HNX-0E and 8 days for HNX-14C. For toxicity and antitumour activity studies, 5-FU pellets containing different amounts of drug (5, 10, 15, 20 mg) were implanted subcutaneously on the back of mice after ether anaesthesia. Care was taken to avoid any direct contact of the pellet with the tumour even when this had grown to its maximum size. Bolus 5-FU was administered i.p. at the MTD: 100 mg/kg i.p. for Balb/c and C57/B1 mice and 125 mg/kg for nude mice. Leucovorin was injected intraperitoneally (i.p.) according to the doses and schedules indicated in the experiments. Treatment was started when the tumours had reached a size of 50-150 mm\textsuperscript{2} (approximately 10 days for Colon 26, 18 days for Colon 38, 16 and 24 days for HNX-14C and HNX-0E, respectively).

\textbf{Enzyme assays}

Tumours were removed at different time points after pellet implantation, or from untreated controls, and were immediately frozen in liquid nitrogen. TS assays, FdUMP binding assay and \textsuperscript{3}H-release assay, were performed as described [17]. The frozen tissues were pulverised by means of a microdisembrator (Braun, Melsungen, Germany) and the frozen powder was
suspended in a Tris–HCl buffer (200 mM) containing 20 mM β-mercaptoethanol to increase the stability of disulphate bonds present in the TS molecule and 100 mM NaF and 15 mM CMP to inhibit the breakdown of nucleotides. Temperature was maintained at 4°C and pH at 7.4. The suspension was centrifuged at 4000 g and the supernatant subsequently at 7000 g. The enzyme-containing suspension was split in several parts and used for different assays. Details have been described previously [17].

Briefly, the first assay was a ligand-binding assay with 6-[3H]-FdUMP as the substrate, used to determine the free FdUMP binding sites of TS. Enzyme suspension (50 μl) was mixed with 30 μl 6.5 mM CH2-THF, 135 μl Tris–HCl buffer and the reaction was initiated by addition of 15 μl 570 nM 6-[3H]-FdUMP; the mixture was incubated at 37°C for 1 h, stopped by addition of 500 μl 10% neutral charcoal, vigorously mixed, chilled on ice and centrifuged. Radioactivity was estimated by liquid scintillation counting of 250 μl supernatant.

The second assay determines the catalytic activity of TS at a final concentration of 1 and 10 μM 5-[3H]-dUMP by means of the 3H-release. The TS catalytic activity assay in control tumours consisted of: 25 μl enzyme suspension (at different dilutions), 5 μl 6.5 mM CH2-THF, 10 μl Tris–HCl or 10 μl 0.05 mM FdUMP to measure the potential inhibition of TS in control samples. The assay was initiated by addition of 10 μl 5-[3H]-dUMP (1 or 10 μM final concentration) and was incubated for 30 min at 37°C, stopped by addition of 50 μl ice cold 35% trichloroacetic acid and 250 μl 10% neutral activated charcoal. After centrifugation, 150 μl of the supernatant was counted by liquid scintillation [17].

Before the assays were performed, endogenous FdUMP and other nucleotides were removed with activated neutral charcoal in samples from treated animals. This charcoal wash was also performed on control samples and did not change the results of the assays. In a number of samples both the total amount of TS and the residual TS activity were measured. For this purpose, assays were only performed after a dissociation step. One part of the enzyme suspension was used for dissociation of FdUMP from TS, by incubation of equal volumes of the enzyme suspension and dissociation buffer (0.75 M NH4CO3, 100 mM NaF, 20 mM β-mercaptoethanol, 1.5 mM CMP, pH 7.8) and 1/20 of the total volume of 1.6 mM dUMP for 3 h at 30°C. The high concentration of dUMP facilitated dissociation, prevented reassociation of FdUMP and stabilised TS. Addition of 10% neutral charcoal (same volume as the total dissociation volume) was used to remove the nucleotides after dissociation. An equal volume of the enzyme suspension was frozen at −70°C and thawed after 3 h. This part of the enzyme suspension was also treated with 10% neutral charcoal. Both assays of TS were performed in the dissociated and non-dissociated samples, enabling determination of the total and free number of FdUMP binding sites, respectively, and the total and residual catalytic activity, respectively. Protein content of the tumours was assayed using the BioRad protein assay.

**Analysis of 5-FU and FdUMP levels**

Plasma concentrations of 5FU were measured in healthy female C57/B1 mice: blood was drawn retro-orbitally between 9.00 a.m. and 10.00 a.m., 3–4 h after light onset (HALO), in order to avoid circadian variability. Differences in concentrations at various time points were studied on day 10, when 5 animals were sampled at six time points from 3 HALO until 15 HALO (8 h dark, 16 h light). Blood was centrifuged and plasma samples were immediately frozen. 5-FU concentration was measured by gas chromatography coupled to mass-spectrometry (GC—MS) as previously described [25, 32]. The sensitivity and selectivity of this analytical procedure enables the use of very small samples (10 μl); in this way every animal could be sampled repeatedly during the 3 weeks and a complete pharmacoKinetic profile was obtained for each animal. In order to exclude the possibility that pellets were exhausted after a few days from implantation, some pellets removed from several mice after 10 days were implanted in different mice and plasma concentration was measured during the following period. 5-FU and FdUMP concentrations were also measured in Colon 26 tumours during the infusion at different times after implantation of the pellet. Tissues were removed during anaesthesia and immediately frozen in liquid nitrogen. 5-FU and FdUMP concentrations were measured as described previously [17, 25].

**RESULTS**

**Toxicity**

The extent of systemic toxicity was expressed as percentage of weight loss following the implantation of the pellet. Pellets containing different amounts of 5-FU were studied in healthy mice: larger pellets (15 and 20 mg) caused an important loss of weight (approximately 25% at day 4) and proved rapidly fatal, while weight loss was 11% at day 11 following implantation of 10 mg pellets (Figure 1). The pattern of systemic toxicity differed from that of bolus injections [28]: weight loss was not seen in the first days after the beginning of treatment, but after 10 days, and the maximum weight loss during continuous infusions was higher than that observed after bolus injection. Similar data on toxicity were obtained in tumour-bearing animals (Table 1).

Pellets containing 10 mg 5-FU, released over 3 weeks were considered to be the MTD for this form of therapy, and these pellets have been used for all the following studies unless otherwise indicated. The additional effect of LV was investigated at various doses and schedules. A weekly bolus injection of 100 mg/kg had no effect, but small daily doses of 5 mg/kg resulted in severe diarrhoea, major weight loss and increased mortality (Table 1).

The pattern of haematological toxicity of 5-FU pellets has been studied in healthy mice at the MTD of 10 mg per mouse (Figure 2a–c). Haematocrit (Figure 2a) reached a nadir of 70%...
of initial values after 7 days and it returned to normal levels after 20 days, coinciding with the end of the infusion. The effect on granulocytes was characterised by a 60% decrease after 4 days compared with pretreatment levels (Figure 2b). However, this was followed by a marked rebound after about 20 days, and by the return to normal levels after discontinuation of the 5-FU release. The initial effect on thrombocytes (Figure 2c) was comparable to that on total white blood cells, but only a moderate rebound was observed. Animals in which only the carrier material of the pellet was implanted and untreated controls showed no significant changes in white blood cell counts or in haemocrit. A late effect of sampling was observed on the return to normal levels after discontinuation of the 5-FU release. The median lifespan of control mice (starting from the day of first treatment) is set at 100%; the number in the parentheses is the number of experiments with at least 6 mice. T/C is given for day 7 for Colon 26 and Colon 38, and day 10 for HNX-OE and HNX-14C; lifespan was not assessed for mice bearing this tumour, they were sacrificed when tumour load exceeded 2000 mm³; significantly different from the bolus injections (test for unpaired samples) at the level of: *0.01 < P < 0.02 (**); **0.001 < P < 0.01 (***).

## Table 1. Antitumour activity of 5-FU bolus and continuous infusions

<table>
<thead>
<tr>
<th>5-FU Dose</th>
<th>LV</th>
<th>Schedule</th>
<th>Max. weight loss</th>
<th>T/C</th>
<th>GDF†</th>
<th>Median lifespan‡ (days)</th>
<th>Increase of lifespan§ (%)</th>
</tr>
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<tbody>
<tr>
<td><strong>Colon 26</strong></td>
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<tr>
<td>100 mg/kg</td>
<td>20.6</td>
<td>Weekly</td>
<td>0.13 ± 0.02 (5)</td>
<td>2.5 ± 0.9 (5) &gt;50 (5)</td>
<td>n.e.</td>
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<tr>
<td>5 mg</td>
<td>10.6</td>
<td>Weekly</td>
<td>0.42</td>
<td>2.9</td>
<td>&gt;46</td>
<td>n.e.</td>
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<tr>
<td>10 mg</td>
<td>10</td>
<td>Weekly</td>
<td>0.59</td>
<td>1.7</td>
<td>47</td>
<td>n.e.</td>
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<tr>
<td>100 mg/kg</td>
<td>7</td>
<td>Weekly</td>
<td>0.59</td>
<td>1.7</td>
<td>47</td>
<td>n.e.</td>
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<td>2</td>
<td>Weekly</td>
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<td>47</td>
<td>n.e.</td>
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<tr>
<td><strong>Colon 38</strong></td>
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<tr>
<td>100 mg/kg</td>
<td>20.6</td>
<td>Weekly</td>
<td>0.13 ± 0.02 (5)</td>
<td>2.5 ± 0.9 (5) &gt;50 (5)</td>
<td>n.e.</td>
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<td>&gt;46</td>
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</table>
Figure 2. Haematological toxicity of 10 mg 5-FU pellets in C57Bl/6 mice: (a) haematocrit, (b) leucocytes, (c) platelets. Values for controls (-□-) and pellet treatment (-∆-) were expressed as a percentage of initial values calculated per individual mouse. Initial values are means ± S.D. of 20 mice: haematocrit, 47.5% ± 3.8%; leucocytes, \(3.9 \times 10^6\) ± 1.1 \(\times 10^6\) cells/ml; platelets, \(1413 \times 10^3\) ± 550 \(\times 10^3\) cells/ml. Values at other time points are means ± S.D. of at least 3 mice. Student's t-test for paired data: (a) haematocrit of treated mice is significantly lower than before treatment at days 4–14 \((P < 0.01)\). (b) After 4–7 days the number of leucocytes of treated mice was significantly lower than before treatment \((P < 0.05)\). At day 18 the number was significantly higher than before treatment \((P < 0.05)\). (c) The number of platelets of treated mice was significantly lower than before treatment at day 4 \((P < 0.05)\). At day 12 it was significantly higher than before treatment \((P < 0.05)\).

We also measured plasma concentrations at different time points during the day after 10 days from the implantation of pellets. A 10-fold difference between the plasma concentration at 3 HALO and the peak value at 6 HALO was consistently observed in each animal. At 12 HALO and later, plasma 5-FU concentrations were generally undetectable (Figure 4b). Peak and trough values were significantly different from the calculated means.

Figure 3. Relative antitumour activity on Colon 26 of 100 mg/kg bolus 5-FU (-□-), 10 mg 5-FU pellets (-△-), 10 mg 5-FU pellets combined with 5 mg/kg LV given as 5 daily bolus injections (-▽-) compared with controls (-△-). Values are means ± S.D. of 3–6 mice (6–12 tumours).

Figure 4. (a) 5-FU plasma concentration in C57Bl/6 mice at 3–4 HALO (mean values ± S.D. of at least three values). (-□-); values from one of the pellets that was implanted in one mouse and after 10 days was moved to another (-●-). (b) Circadian variation studied on day 10 from pellet implantation, data are shown for each separate animal. Time is measured as hours after light onset (HALO); 3 HALO corresponds to 9.00 a.m. Student's t-test for paired data: plasma concentration at 12.00 (6 HALO) was significantly different from all other time points \((P < 0.01)\).
During the infusion we also measured 5-FU concentration in tumours (Figure 5). The highest concentrations were observed at the beginning of the infusion. At all time points 5-FU concentrations in the tumour were at least 2-fold higher than the corresponding levels in plasma.

**FdUMP concentrations and effect on TS**

The concentrations of FdUMP were measured in tumours at various time points after implantation of the pellets. However, at all time points the FdUMP concentrations were below the detection limit (12 pmol/g wet weight) of the assay. Despite the low levels of FdUMP, the activity of TS was inhibited significantly during the first week (Figure 6a, b). The number of free FdUMP binding sites was reduced significantly at days 4 and 7 after the start of treatment. However, after 11 days a partial recovery was observed, followed by a complete recovery to normal levels after 2 weeks. The catalytic activity of TS showed a somewhat different pattern with a small decrease in the residual TS activity after 4 and 7 days and a comparable total TS catalytic activity. After 11 days a 3-fold increase in TS residual catalytic activity was accompanied by an almost 5-fold increase in the total catalytic activity. Thereafter, the total catalytic activity decreased and was comparable on the residual activity after 14 days.

**DISCUSSION**

Continuous infusion of 5-FU has a considerable interest in the clinic. In this paper we show that it is feasible to administer 5-FU continuously over 3 weeks to mice attaining plasma levels that are comparable to those observed during continuous infusions in patients [33–38] and in rats [39], although in the latter species, and in some of the studies in patients, infusions were shorter. During limited infusion periods (3–7 days), plasma concentrations tended to be higher than in longer infusions (21 days) possibly due to the higher dose which could be administered. The overall results, both antitumour activity and toxicity, are also in agreement with the clinical data [7, 8], showing the validity of the murine model system. Clinical studies report a higher response rate for continuous infusion than for bolus injections, but the overall results in terms of survival are not different. The plasma and tumour concentrations in mice are in the range of the concentrations which cause cell killing in vitro [11]. In a cell line derived from Colon 26, C26-10, the IC50 for 5-FU, 72 h exposure, was 0.6 µM, at which a significant TS inhibition was observed. It should be noted that at longer incubations in vitro the IC50 values for 5-FU decreased. Our in vitro results demonstrate that continuous infusion was more effective than bolus treatment in the initial inhibition of tumour growth, but this did not lead to a longer survival of the animals.

From the concentration versus time plot it appeared that the profile of 5-FU concentrations in plasma was comparable to that observed in patients, but very different from that of bolus injections in which peak concentrations between 500 µM and 1 mM are observed. The somewhat higher concentration just after the implantation of the pellet (0.2 µM compared with 0.05–0.1 µM at later time points) was possibly due to the process by which pellets start to release the drug before reaching a stable rate of delivery. Only after 16 days did we observe a significant decrease in plasma levels. The increase in FdUMP binding sites in tumours, the rebound in blood cells and the increased rate of

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**Figure 6.** (a) FdUMP binding to TS in Colon 26 tumours at different time points after pellet (10 mg 5-FU) implantation. Control (open bars), free FdUMP binding sites after treatment, measured without dissociation (closed bars), total FdUMP binding sites after treatment measured after dissociation of samples (hatched bars). Values are means of at least 3 tumours ± S.D. Student's t-test unpaired data: at 4, 7 (P < 0.001) and 11 days (P < 0.05) after the start of 5-FU treatment the total FdUMP binding was significantly lower than control. A significant difference between the number of free binding sites and total binding sites was only observed at day 12 (P < 0.01). (b) TS catalytic activity of Colon 26 tumours at different time points after pellet (10 mg 5-FU) implantation. Control (open bars), residual TS catalytic activity after treatment, measured without dissociation (closed bars), total catalytic activity after treatment measured after dissociation of samples (hatched bars). Values are means of at least 3 tumours ± S.D. Student's t-test unpaired data: no significant differences were observed between residual and total TS catalytic activity, but the total TS catalytic activity on days 11, 12 and 14 was significantly higher than the control value (P < 0.001).
tumour growth were already observed on day 7, well before the
increase in plasma and tissue levels of 5-FU. The small trough
apparently present after 10 days was not significant. Exhaustion
of the pellet is unlikely at that time point since animals that
received pellets that had been implanted in different mice for 10
days showed similar plasma concentrations of 5-FU (Figure 4a).
Variations in plasma concentration could also be due to changes
in metabolism. These may consist of an increase in the activity
of catabolising enzymes with time resulting in decreased plasma
5-FU levels despite a regular delivery from the pellet. These
changes in 5-FU plasma concentrations, however, were smaller
than the variations observed during continuous infusion using
pumps in patients [40]. Owing to these variables we did not
include a pharmacokinetic evaluation. More extensive sampling
would be required for a proper pharmacokinetic evaluation, and
this would be impossible in the same mouse both for ethical an
methodological reasons.

Plasma 5-FU concentrations measured at 3 HALO corre-
sponded to the values seen in the animals sampled over the entire
3 weeks. Plasma samples obtained at later points of the day show
that 5-FU concentrations increased to reach a peak at 6 HALO,
followed by a decline. These data are in agreement with the
circadian rhythm of DPD observed in rat liver [21] which is
the main tissue responsible for 5-FU elimination [1, 2]. The
variation in enzyme activities is possibly related to the different
antitumour activity of 5-FU when the drug is administered at
different time points [41] and to the presence of varying 5-FU
plasma concentrations during continuous infusion in patients
[18]. These concepts are currently being explored in the clinic
and “chronomodulated ” delivery of 5-FU and of other drugs is
becoming increasingly popular [24, 42-44].

In terms of dose delivered, continuous infusions offered a
deri se concentration than bolus injections. The total amount of
drug administered was 10 mg versus 6 mg. This was comparable
with the higher dose of 5-FU that can be given to patients with
continuous infusion using schedules varying from 3 days to
several weeks [7, 8, 33-38].

The pattern of haematological toxicity induced by continuous
infusion was quite different from what was observed with bolus
injections. Nadir values observed after bolus injections were
lower in the same strain of mice (20%) [28, 29], but were reached
later, while the rebound was lower and only observed after discon-
tinuation of the treatment. In continuous infusion the
rebound was even observed during infusion which would indi-
cate that the low concentration of 5-FU might affect differen-
tiation/proliferation of primitive stem cells. Evidence has indeed
been obtained that low 5-FU concentrations can be immuno-
stimulating [45] and the analysis of blood smears indicated that
granulocytes were more affected than lymphocytes (data not
shown). Different effects were also observed for the haematocrit
(more severe after bolus injections). The data correspond to the
observed lack of myeloid toxicity in the clinic with protracted
infusion of 5-FU [7-9].

In contrast to the haematological toxicity, weight loss at the
MTD was maximal 11 days after pellet implant, much later than
what was observed with bolus injections [28]. This difference in
the time course of systemic toxicity was particularly evident
when, during the determination of the MTD, mice were treated
with doses of 5-FU that proved lethal. When an excessive dose
was given as an infusion, weight loss was rapid and death
occurred within 1 week (Figure 1). This is consistent with
experimental and clinical observations on the different toxicity
seen after bolus or infusional administration of 5-FU: mainly
haematological for bolus treatment, gastrointestinal for
infusions. We have observed that a bolus injection of 5-FU can
inhibit TS in mucosal tissue [46] and the prolonged presence of
5-FU might be particularly toxic to gastrointestinal tissues.
LV administration enhanced the gastrointestinal toxicity of
infusional 5-FU, suggesting that TS inhibition was involved.
The continuous, repeated administration of LV possibly pro-
vided enough folates in these normal tissues in order to enhance
this inhibition, despite the low activity of folypolyglutamate
synthetase in these tissues [47]. The importance of this enzyme
in mouse and to investigate the
association with modulating agents such as interferons.

1. Pinedo HM, Peters GJ. 5-Fluorouracil: biochemistry and pharma-


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