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Systemic exposure of floxuridine after hepatic arterial infusion pump chemotherapy with floxuridine in patients with resected colorectal liver metastases

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ABSTRACT

Background: Floxuridine's high hepatic extraction ratio and short elimination half-life allows maximum liver exposure with minimal systemic side-effects. This study attempts to quantify the systemic exposure of floxuridine.

Methods: Patients undergoing continuous hepatic arterial infusion pump (HAIP) floxuridine after resection of colorectal liver metastases (CRLM) in two centres underwent six cycles of floxuridine at start dose 0.12 mg/kg/day. No concomitant systemic chemotherapy was administered. Peripheral venous blood samples were drawn during the first two cycles: pre-dose (only in the second cycle), 30 min, 1 h, 2 h, 7 h, and 15 days after floxuridine infusion. Foxuridine concentration in the residual pump reservoir was measured on day 15 of both cycles. A floxuridine assay with a lower boundary of detection of 0.250 ng/mL was developed.

Results: 265 blood samples were collected in the 25 patient included in this study. Floxuridine was mostly measurable at day 7 and day 15 (86 % and 88 % of patients respectively). The median dose corrected concentrations were 0.607 ng/mL [IQR: 0.472–0.747] for cycle 1 day 7, 0.579 ng/mL [IQR: 0.470–0.693] for cycle 1 day 15, 0.646 ng/mL [IQR: 0.463–0.8546] for cycle 2 day 7, and 0.534 ng/mL [IQR: 0.4257–0.7075] for cycle 2 day 15. One patient had remarkably high floxuridine concentrations reaching up to 44 ng/mL during the second cycle, without a clear explanation.

The floxuridine concentration in the pump decreased by 14.7 % (range 0.5 %−37.8 %) over a period of 15 days (n = 18).

Conclusion: Overall, negligible systemic concentrations of floxuridine were detected. However, remarkably increased levels were detected in one patient. Floxuridine concentration in the pump decreases over time.

Abbreviations: 5-FU, 5- fluorouracil; AST, Aspartate aminotransferase; CRLM, Colorectal liver metatases; CTCAE, Common terminology criteria for adverse events; DPYD, Dihydropyrimidine dehydrogenase; GDA, Gastroduodenal artery; HAIP, Hepatic arterial infusion pump; IQR, Interquartile range; IU, International units; LC-MS/MS, Liquid vhromatography tandem mass spectrometry; LLQ, Lower limit of quantitation; MAA, Macroaggrgated albumin; MRM, Multiple reaction monitoring mode; PK, Pharmacokinetics; SD, Standard deviation; UPLC, Ultra high performance liquid chromatography.

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1. Introduction

Adjuvant hepatic arterial infusion pump (HAIP) chemotherapy with floxuridine is a promising treatment for resectable colorectal liver metastases (CRLM) with the aim to improve survival by reducing the rate of liver recurrence [1]. In a large propensity score analysis, the survival benefit was up to three years in patients who received HAIP chemotherapy for resectable CRLM compared with those who did not [2]. Our recent phase II trial with 29 patients who underwent adjuvant HAIP chemotherapy for CRLM showed hepatic toxicity warranting dose reductions in 79 % of patients, but no extrahepatic toxicity (EUCTR2016–004299–24-NL).

The rationale for HAIP chemotherapy is that CRLM primarily derive their blood supply from the hepatic artery rather than the portal vein. The most commonly used intra-arterial agent for adjuvant HAIP chemotherapy is floxuridine, a fluorinated pyrimidine [1]. When given by rapid intra-arterial injection, floxuridine is catabolized to 5-fluorouracil (5-FU), thus, producing similar toxic and antimetabolic effects as 5-fluorouracil. Floxuridine is metabolized in the liver with a reported hepatic extraction ratio of 95 % and a half–life of 8–10 min [3]. In addition, floxuridine has shown linear kinetics, i.e., a linear relation between dose and serum concentration [4]. These properties theoretically allow for a high hepatic dosage while limiting systemic exposure and therefore systemic side effects. Ensminger et al. reported a intratumoral drug concentration with floxuridine reaching 400–fold of that achieved with systemic administration of 5-FU [4].

Although HAIP floxuridine theoretically would lead to a minimal systemic exposure, no previous study was able to quantify the very low amount of floxuridine that passes the liver, in particular in patients who did not receive concomitant systemic chemotherapy. Extra-hepatic symptoms could develop in patient who have increased systemic exposure of floxurudine. It would therefore be valuable to develop a new method to quantify the low floxuridine plasma concentration after hepatic arterial pump infusion at different moments in time to evaluate the pharmacokinetic profile of intra-arterial floxuridine pump infusion. These new insights could aid researchers and treatment physicians alike in a deeper understanding of the biological mechanism behind the clinical effect of continuous intrahepatic administration of floxuridine.

The current study aimed to develop an assay and to measure plasma concentrations of floxuridine in patients undergoing adjuvant HAIP floxuridine after resection of CRLM and correlate the measured exposure to systemic toxicity.

2. Methods

2.1. Study design

This pharmacokinetic (PK) study is a side-study of the multicentre randomised controlled PUMP trial (International Clinical Trials Registry Platform Search Portal (trialsearch.who.int); number NTR7493). In the PUMP trial patients with resectable CRLM are randomised to either resection only (control group) or resection plus adjuvant HAIP chemotherapy with floxuridine (intervention group) [5]. The PUMP trial, including the current pharmacokinetic side-study, was approved by the Erasmus MC medical ethics committee.

2.2. Patient selection

The first 25 consecutive patients randomised into the intervention group of the PUMP trial at the Erasmus MC Cancer Institute (Rotterdam, the Netherlands), the Netherlands Cancer Institute (Amsterdam, the Netherlands) and IJsselland hospital were included in the current PK analysis [5]. Patients with histologically confirmed colorectal cancer and resectable CRLM without extrahepatic disease were eligible. All patients underwent laboratory assessment to confirm an adequate blood count, liver- and renal function. Patients with dihydropyrimidine dehydrogenase (DPD) enzyme function deficiency (based on *DPYD* genotyping) were excluded [6]. A detailed description of the inclusion criteria was described previously [7]. All patients provided written informed consent for the PUMP trial and PK analysis.

2.3. Perioperative procedures

The surgical procedure was previously described in detail [7]. Briefly, all patients underwent surgical resection and/or ablation of CRLM by laparotomy, with or without resection of the primary tumour. At the same time an infusion pump (IP2000V, Tricumed, Kiel, Germany) with a constant non-programmable flow rate of 1.5 mL per day was implanted subcutaneously in the abdominal wall. Cholecystectomy was performed to avoid HAIP chemotherapy-induced cholecystitis [8]. The HAIP catheter was implanted in the gastroduodenal artery (GDA). Before implantation, the pump was filled with a heparinized saline solution (35,000 IU heparin in 35 mL NaCl 0.9 %). Prior to the start of HAIP chemotherapy, a technetium-99m-labeled macroaggregated albumin ($^{99\ m}$Tc-MAA) scintigraphy was performed to reconfirm absence of extrahepatic perfusion and presence of bilobar perfusion, as previously described [7]. Pump refills with the heparinized saline solutions were scheduled every two weeks until the start of HAIP chemotherapy with floxuridine.

2.4. Chemotherapeutic regimen

HAIP chemotherapy was initiated 4–12 weeks after surgery upon recovery of the patients' medical condition and bilirubin levels were ≤25 mmol/mL (≤1.5 mg/dL). All patients were scheduled for six treatment cycles. A cycle consisted of two weeks of continuous HAIP infusion with floxuridine followed by two weeks of continuous HAIP infusion with a heparinized saline solution. Floxuridine dosages were calculated according to the Memorial Sloan Kettering Cancer Center protocol (i.e. 0.12 mg/kg per day) [9,10]. Floxuridine was administered in a solution of 35,000 IU heparin and 25 mg dexamethasone in NaCl 0.9 % with a total volume of 35 mL. No concomitant systemic chemotherapy was administered according to Dutch guidelines.

The first cycle was given at 100 % dose, i.e., 0.12 mg/kg/day, in all patients. Subsequent HAIP floxuridine doses were reduced to 80 %, 50 %, or 0 % of the previous dosage in the event of prespecified increased liver values (i.e., aspartate transaminase (AST), alkaline phosphatase (Alk Phos), or bilirubin), as previously described [5], or for other reasons at the discretion of the treating physician.

2.5. Blood sample collection

Blood for the PK analyses was drawn during the first two cycles on the day of floxuridine administration (after 30 min, 1 h and 2 h), halfway through the floxuridine administration (day 7) and on the last day of floxuridine administration and the pump is refilled with heparinized saline solution (day 15). In addition, a pre-dose sample was taken on the first day of the second cycle. The blood samples were collected in 4 mL lithium heparin tubes and centrifuged at 2000 * g (4 °C) for 10 min. At least 1 mL plasma was extracted into a propylene tube and stored at −80 °C until further analysis was performed.

2.6. Assay development and pharmacokinetic analysis

Floxuridine concentrations were measured using a validated liquid chromatography method coupled to a triple quadrupole mass spectrometer (UPLC-MS/MS). Aliquots of 30 μL of plasma samples for the quantitation of floxuridine were extracted by the addition of 10 μL of an Internal Standard Solution (100 ng/mL floxuridine-^{13}C,$^{15}N_2$) in demi water and 1,5 mL ethyl acetate. After vigorously mixing for 10 min and centrifugation for 10 min at 18,000 *g, 1 mL of the organic phase was evaporated at T = 60 °C under a stream of nitrogen. The residues were

resuspended in 75 μL aliquots of 0.02 % formic acid, from which aliquots of 25 μL were injected into the UPLC-MS/MS-system. The column effluent was monitored using the multiple reaction monitoring mode (MRM).

Peak area ratios of floxuridine versus the Internal Standard were a linear function of the concentration from 0.250 to 50.0 ng/mL. The lower limit of quantitation (LLQ) was validated at 0.250 ng/mL.

For floxuridine, the within and between-run precisions at five tested concentrations, including the LLQ, were ≤8.11 % and ≤7.49 %, respectively, while the average accuracy ranged from 93.5 % to 104.4 %. Also, the PK samples below the LLQ were included in the calculation of the median floxuridine concentration. The details of the developed assay con be found in the supplementary files.

In addition, serum 5-FU concentrations were measured in the samples of one patient with remarkably high systemic floxuridine concentrations. The bioanalytical method to quantify 5 FU in lithium heparinized plasma is described by van Doorn et al. [11]. Calibration Curve of this assay is linear over the concentration range of 1.0–100 ng/mL.

2.7. Floxuridine concentration in residual infusion fluid

The residual volume of floxuridine concentration in the pump at day 15 of the first two cycles was measured for the patients treated at the Erasmus MC Cancer Institute. The residual volume was collected into a syringe and stored at T < −80 °C until further analysis was performed. The concentration of floxuridine in the residual floxuridine solution was measured using the developed LC-MS/MS assay by dilution of the residual solution in blank human plasma.

2.8. Clinical outcomes

Surgical outcomes, results of the postoperative ^{99m}Tc-MAA imaging and floxuridine-related toxicity according to Common Terminology Criteria for Adverse Events (CTCAE, version 4.03) were recorded and correlated with results of the pharmacokinetic analysis. Hepatic toxicity was defined as elevation of liver enzymes with or without symptoms. Systemic toxicity was defined as adverse events not attributed to hepatic toxicity.

2.9. Statistical analysis

Continuous variables were presented as medians with interquartile range [IQR] and categorical variables as proportions. Paired t-test was used to test differences in mean dependent variables. Linear regression was used to identify variables associated with a dependent outcome. Logistic regression was used to identify variables associated with a dichotomous outcome. Early hepatic toxicity was defined as hepatic toxicity warranting a dose reduction for cycle 2. All analyses were performed using SPSS version 24.0 (SPSS Inc., Chicago, IL) and R version 3.5.1 (http://www.r-project.org).

3. Results

3.1. Patient characteristics

The first 25 evaluable patients were included in this study within the period of January 2019 and October 2020. Table 1 provides an overview of the baseline characteristics. None of the patients had extrahepatic perfusion or increased lung shunt upon postoperative ^{99m}Tc-MAA. Six patients (24 %) received neo-adjuvant systemic chemotherapy at least two months before the first floxuridine cycle with a median time between neo-adjuvant chemotherapy and the first floxuridine cycle of three months (median= 2.3, IQR 2.3–3.0).

Fig. 1 summarises the treatment cycles per patient. All patients started at 100 % floxuridine dose (median 227 in the pump, IQR: 177–256 mg) according to the study protocol. During the second cycle, eight patients (32 %) underwent dose reduction to 80 % (n = 4), 50 % (n = 2), or 25 % (n = 1) due to increased liver enzymes. One patient skipped the second floxuridine cycle due to increased liver enzyme disturbances, and restarted at 25 % of the first dose during the 3rd cycle; the blood samples for the second cycle were therefore postponed to the third cycle for this patient. The median amount of floxuridine in the pump for the second cycle was 188 mg (IQR: 138–240 mg). Three patients underwent the maximum dosage of floxuridine (6 cycles at 100 %). The remaining 22 patients underwent one or multiple dose reductions due to increased liver enzymes (n = 18), disease recurrence (n = 3), abdominal pain (n = 2) and/or the patients' wish (n = 2).

Table 1
Baseline characteristics.

Patient characteristics (n = 25)	n (%)
Age at resection (years), median [IQR]	60 [57–70]
Sex (female)	12 [48]
Weight, median [IQR]	88 [69–98]
Body mass index, median [IQR]	28 [24–31]
Number of CRLM, median [IQR]	1[1,2]
Largest diameter CRLM (cm), median [IQR]	2.2 [1.8–2.9]
Neo-adjuvant chemotherapy	6 [24]
CAPOX	1 [17]
CAPOX Bevacizumab	3 [50]
FOLFOX	2 [33]
Interval last CTx to first FUdR cycle (months), median [IQR]	3 [2.3–3.0]
Treatment outcomes	
Major hepatectomy[a]	3[12]
Synchronous resection	7 [28]
Increased lung shunt on postoperative SPECT/CT	0 (0)
Weeks from surgery to first Floxuridine Cycle, median [IQR]	5[4–6]
Full dose in pump (mg), median [IQR]	227 [177–256]

IQR: interquartile range; CRLM: colorectal liver metastases; CAPOX: Capecitabine and Oxaliplatin Neo-CTx: neoadjuvant systemic chemotherapy; FOLFOX: 5 Fluorouracil and Oxaliplatin; FUdR: floxuridine.
[a] Resection of 3 or more adjacent liver segments

3.2. Peripheral venous blood samples

Blood samples at eleven different points in time were scheduled for all 25 patients, aiming for a total of 275 PK samples. Table 2 summarises the number of PK samples collected at each time point. During the study, 265 PK samples were collected (96 % of target number, 10 missing samples). Most missing samples were scheduled on day 7 after the floxuridine infusion (n = 5) and were missed due to patients' refusal to make the extra hospital visit on day 7 for the sole purpose of sample collection for the study. Furthermore, one patient missed 3 samples after the first floxuridine infusion, one patient missed a sample at day 15 after the second floxuridine infusion, and one sample was collected but contained not enough plasma for the analyses.

3.3. Pharmacokinetic analysis

Floxuridine could be measured in 103 PK samples (39 %, Table 2). The floxuridine concentration was below the LLQ of 0.250 ng/mL for the other 162 samples (61 %). Fig. 1 illustrates all floxuridine concentrations at the different points in time. The highest concentrations were measured at day 7 and day 15 after floxuridine infusion. The dose corrected concentrations were 0.607 ng/mL [IQR: 0.472, 0.747] for cycle 1 day 7, 0.579 ng/mL [IQR: 0.470, 0.693] for cycle 1 day 15, 0.646 ng/mL [IQR: 0.463, 0.8546] for cycle 2 day 7, and 0.5340 ng/mL [IQR: 0.4257, 0.7075] for cycle 2 day 15.

Fig. 2 presents box plots showing decreasing concentrations of floxuridine in peripheral venous blood between days 7 and day 15 of the first floxuridine cycle. The median concentration dropped from 0.65 ng/mL (IQR: 0.47–0.79) at day 7–0.54 ng/mL (IQR: 0.41–0.66) at day 15 (i.e. 17 % decrease).

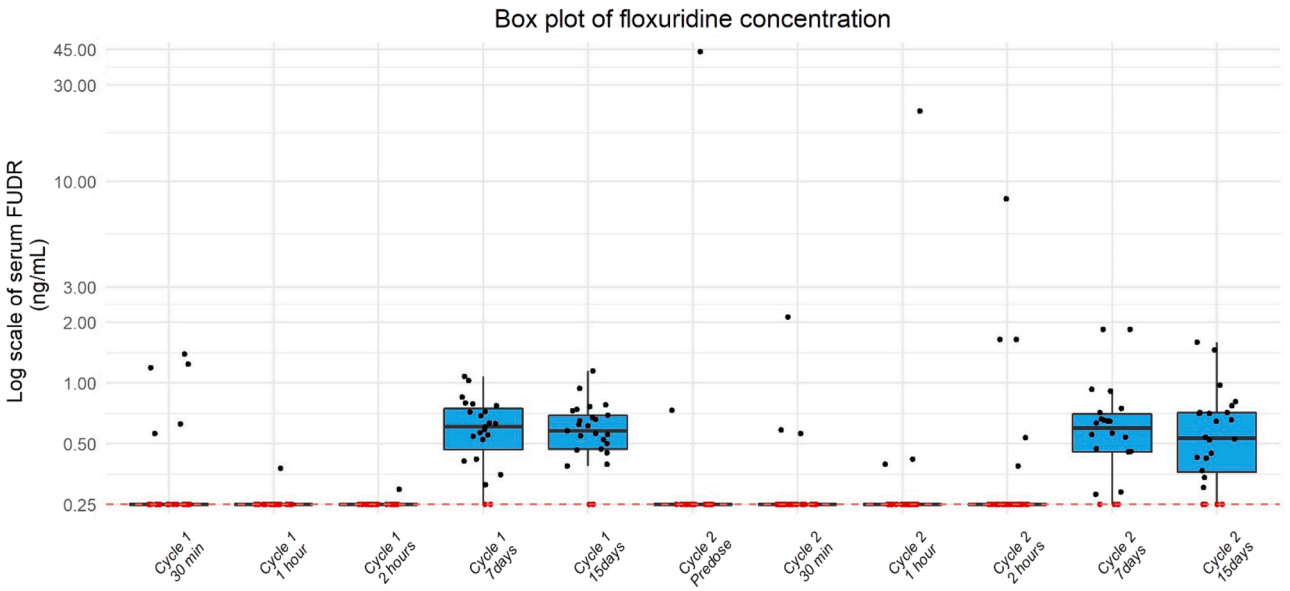

Fig. 1. Floxuridine concentrations measured at each time point. Box plot summarizing the measured concentrations of systemic floxuridine. The black dots represent serum floxuridine concentration measurements above the detection threshold; the red dots represent serum floxuridine concentrations under the detection threshold (at 0.25 ng/mL or 0.5 ng/mL). The median and interquartile ranges were plotted at the 0.25 ng/mL point if they fell under the detection threshold.

Table 2
Overview of pharmacokinetic samples.

Cycle	PK moment	No. of collected samples	No. of measured floxuridine concentrations	No. of samples below the detection border	Dose corrected floxuridine concentration (median ng/mL, (IQR))[a]
Cycle 1	30 min	23	5	18	-
	1 h	24	1	23	-
	2 h	24	1	23	-
	7 days	23	21	2	0.69 (IQR 0.55, 0.79)
	15 days	25	23	2	0.55 (IQR 0.45, 0.52)
Cycle 2	pre-dose	25	2	23	-
	30 min	25	3	22	-
	1 h	25	3	22	-
	2 h	25	5	20	-
	7 days	22	19	3	0.65 (IQR 0.46, 0.85)
	15 days	24	20	4	0.53 (IQR 0.42, 0.71)
Total no. of samples		265	103	162	-

[a] Dose corrected concentrations are only depicted for day 7 and day 15, since the number of measured concentrations on the other time points was considered too low.

In one patient remarkably high systemic floxuridine concentrations were measured in one patient during the second floxuridine infusion cycle (Fig. 3). The highest floxuridine concentration was measured at the pre-dose sample (before the start of cycle two) 44 ng/mL. After the second floxuridine infusion, the floxuridine concentration in this patients was 2.12 ng/mL after 30 min, 22.3 ng/mL after 1 h, and 8.18 ng/mL after 2 h. At day 7 the floxuridine concentration was 0.741 ng/mL in the first and 1.59 ng/mL in second cycle. Floxuridine concentrations during the first cycle were between < 0.250 ng/mL (under the LLQ) and 1.19 ng/mL, comparable to other patients.

3.4. Floxuridine concentration in residual infusion fluid

The mean calculated floxuridine concentration administered in the pump reservoir on day 1 was 5.11 mg/mL (SD: 1.28) for the first cycle and 4.38 mg/mL (SD: 1.59) for the second cycle. The residual floxuridine concentrations in the infusion pumps were measured for the first two cycles in 18 patients. The mean decrease in concentration between the administered floxuridine concentration and the measured concentration in the residual fluid in the pump after 15 days was 14.7 % (range 0.5–37.8 %).

3.5. Adverse events during HAIP floxuridine regime

Table 3 summarises all adverse events that occurred since the first floxuridine cycle until two weeks after the last refill of the infusion pump. Floxuridine-induced elevated enzymes warranting dose reduction (CTCAE grade 2) occurred in 18 patients and were due to elevated Alk Phos (n = 15), AST (n = 10) and/or bilirubin (n = 3). Two patients had floxuridine-induced hyperbilirubinemia and biliary sclerosis requiring endoscopic stent placement (CTCAE grade 3).

Twelve patients had a total of 16 systemic adverse events of CTCAE grade 1 (n = 9), CTCAE grade 2 (n = 3) and/or grade 3 (n = 4) (Table 3).

4. Discussion

This is the first study quantifying very low floxuridine concentrations in peripheral venous blood after HAIP floxuridine in patients who underwent HAIP floxuridine without concomitant systemic chemotherapy after resection of CRLM. The results confirm overall low systemic exposure of floxuridine, and none of the patients had systemic side-effects that could be attributed to extrahepatic exposure of floxuridine.

Previous studies developed assays for floxuridine. In 1978, Ensminger et al. used a radioimmunoassay specific for floxuridine to measure systemic floxuridine concentrations in 15 patients with liver cancer receiving hepatic arterial infusion of floxuridine [3]. The lower boundary of limit of quantification of this radioimmunoassay method was 0.02 μM, corresponding to 4.92 ng/mL. Tsume et al. developed a mass spectrometry method to measure floxuridine with a lower limit of quantification for floxuridine of 50.0 ng/mL [12]. However, Tsume

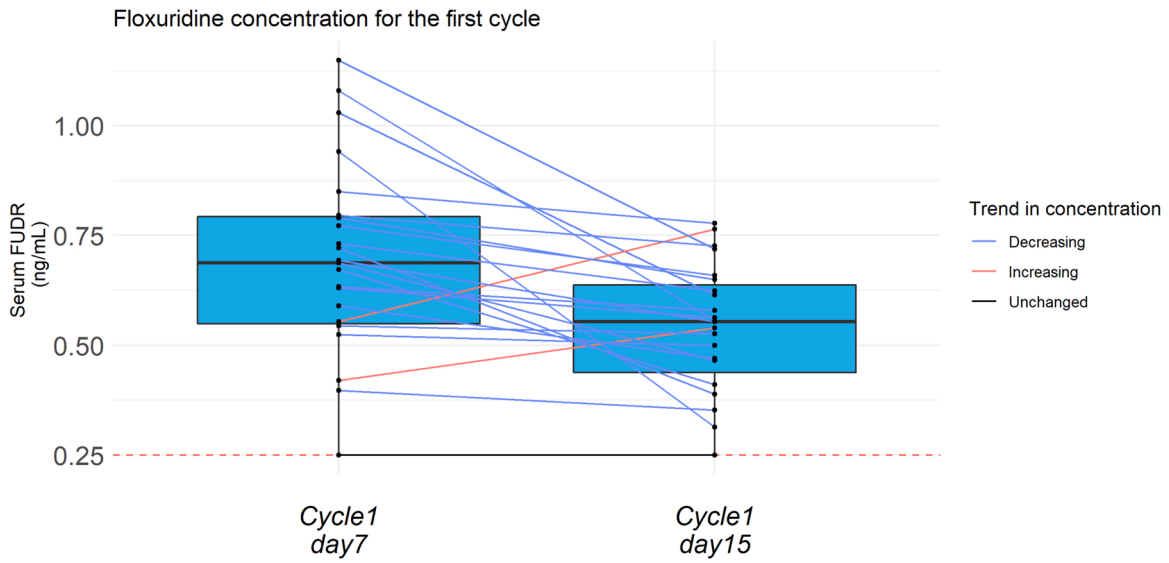

Fig. 2. Box plots showing decreasing concentrations of floxuridine in peripheral venous blood between days 7 and day 15 of the first floxuridine cycle. Most patients showed a decrease in floxuridine levels between Cycle 1 day 7 and Cycle 1 day 15, which is consistent with the potential degradation over time observed in the residual volume analysis.

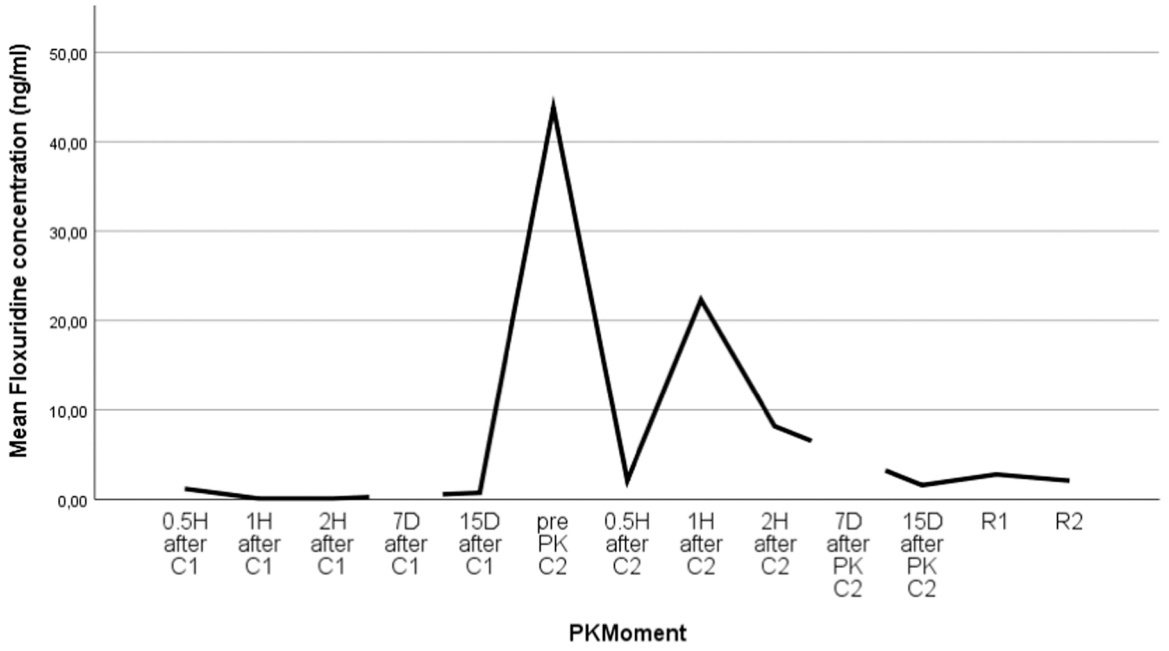

Fig. 3. Floxuridine concentrations of the one patient with remarkably high serum concentrations of floxuridine.

et al. did not use this assay to measure floxuridine serum concentrations after hepatic arterial infusion. Since the systemic concentration of floxuridine is expected to be very low due to the high first pass effect, the lower boundary of limit of quantification of both these assays is too high to measure floxuridine in peripheral venous blood after hepatic arterial infusion.

The UPLC-MS/MS assay developed in the current study is the first method that is able to quantify the very low floxuridine concentrations of floxuridine in peripheral blood that were measured after HAIP chemotherapy with floxuridine. The lower boundary of detection of this method was 0.250 ng/mL, which is significantly lower than the previously developed methods by Tsume et al. (i.e. 50 ng/mL) [12]. or Ensminger et al. (i.e. 4.93 ng/mL) [3]. The results of the current study give insights in the pharmacokinetic profile of floxuridine administered as HAIP floxuridine.

Due to the previously reported high hepatic extraction by the liver and short elimination half-life [3], the overall low peripheral blood concentration and low number of systemic side effects were in line with expectations. The recorded systemic side-effects are common post-operative complications or in line with the course of the disease. However, relation with systemic floxuridine cannot be excluded and should be investigated further.

Serum floxuridine concentrations at day 15 were slightly lower than on day 7 for both cycles in all patients. This could be explained by gradual degradation of floxuridine in the pump. At the end of the two-week infusion, the floxuridine concentration of the residual fluid in the pump reservoir was 15 % lower than concentration that was administered on day 1. However, the assay was developed to measure

Table 3
Adverse events since start floxuridine treatment until 2 weeks after the infusion pump was lastly filled.

	CTCAE grade 1	CTCAE grade 2	CTCAE grade 3
Hepatic toxicity			
Elevated Alk Phos	-	15	-
Elevated AST	-	10	-
Elevated bilirubin (no sclerosis)	-	3	2
Biliary sclerosis	-	-	2
Systemic toxicity			
Nausea	2	-	-
Ileus	-	-	2
Fatigue	1	-	-
Anxiety	1	-	-
Pulmonary embolism	-	-	2
Fever	1	-	-
Urinary tract infection	-	1	-
Abdominal pain	1	1	-
Gastritis	-	1	-
Anemia	3	-	-

Alk Phos: Alkaline Phosphatase; AST: Aspartate Aminotransferase

serum concentrations, and not the concentration in the prescribed floxuridine solution. In addition, the assay was not used to measure the initial concentration of floxuridine on day 1 of the cycle. Nevertheless, previous studies using the same HAIP floxuridine treatment protocol showed promising results despite potential degradation of floxuridine. [1] However, the potential degradation of floxuridine inside the pump should be researched further.

One patient was an outlier with increased systemic floxuridine levels reaching up to 44 ng/mL. The time points at which peak 5-FU concentrations measured in this patients coincided with those of peak floxuridine concentrations. However, the highest 5-FU concentration measured (19.2 ng/mL) was only 10 % of that described in patients receiving systemic treatment with 5 -FU at 2000 mg and 3500 mg daily, making systemic toxicity due to increased 5-FU and floxuridine unlikely [11]. This patient indeed had no systemic adverse events. Unexpectedly, the highest concentrations of floxuridine in this patient were measured just before the second floxuridine infusion and 1 and 2 h after administration of cycle 2. These were highly unexpected time points to have peak systemic floxuridine concentrations for which we have no plausible explanation. An error in the collection of the PK samples could not be identified afterwards. Drug accountability and bioanalysis showed no inconsistencies. Scintigraphy was performed to reconfirm absence of extrahepatic perfusion (results not shown). One would expect higher concentrations at all different points in time if potential genetic variants in drug transporters or enzymes would have played a role. Although, the other concentrations of cycle were consistently higher, the measured concentrations in cycle 1 were at some points even below the limit of quantification. Any influence of genetic variants seems therefore unlikely.

Floxuridine is a prodrug of 5-FU, which is the active metabolite responsible for the therapeutic effects of floxuridine.[3] Therefore, even assuming a 100 % conversion rate to 5-FU, the overall plasma concentrations of systemic floxuridine can be considered negligible, as they do not contribute significantly to the overall pharmacological activity of the drug.

Strengths of this study were the development of a sensitive UPLC-MS/MS method, which were validated to measure floxuridine concentrations. Additionally, multiple blood samples were taken during two cycles of floxuridine as part of randomized controlled trial. Moreover, floxuridine residual levels of the pump were measured at day 15, suggesting potential degradation during the two-wee infusion period. Lastly, no concomitant systemic chemotherapy was administered with HAIP floxuridine, thus removing any potential interactions with other chemotherapeutic agents, in particular 5-FU.

Nonetheless, this study is limited by a small sample size, and therefore no analysis could be made to investigate risk factors for increased serum floxuridine or correlation between systemic floxuridine concentrations and symptoms not related to hepatotoxicity.

In conclusion, negligibly low systemic concentrations of floxuridine were measured after HAIP chemotherapy with floxuridine in this PK study. The highest concentrations were mostly measured halfway the floxuridine cycles, i.e., on days 7 and 15 of the HAIP floxuridine cycles, as expected. Increased serum concentrations occurred in one patient, but no floxuridine-attributed extrahepatic toxicity was observed. The residual floxuridine concentration in the pump was 15 % lower than on day 1 when the pump was filled, suggesting potential degradation of floxuridine in the pump during the two–week infusion period.

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CRediT authorship contribution statement

N Ijzerman: Formal analysis, Investigation, Writing - review & editing, Project administration, **WF Filipe:** Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Project administration, **P de Bruijn:** Conceptualization, Software, Formal analysis, Data curation, Writing - review & editing, **FE Buisman:** Investigation, Writing - review & editing, Project administration, **L van Doorn:** Investigation, Writing - review & editing, Project administration, **P Doornebosch:** Investigation, Writing - review & editing, **J Holster:** Investigation, Writing - review & editing, Project administration, **C Grootscholten:** Investigation, Resources, Writing - review & editing, **DJ Grünhagen:** Investigation, Writing - review & editing, **CPE van Bommel:** Investigation, Writing - review & editing, **MYV Homs:** Investigation, Writing - review & editing, **N Kok:** Investigation, Resources, Writing - review & editing, Supervision, **C Verhoef:** Investigation, Resources, Writing - review & editing, Supervision, Funding acquisition, **B Groot Koerkamp:** Conceptualization, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition, **KFD kuhlman:** Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition, **RHJ Mathijssen:** Conceptualization, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition, **SLW Koolen:** Conceptualization, Software, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Conflict of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Part of a ongoing RCT, data is stil confidential until primary endpoint (survival) results are published.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.114625.

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