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ORIGINAL ARTICLE

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Late-onset delayed excretion of methotrexate

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Abstract Pleural effusions, ascites, and renal dysfunction decrease the plasma excretion of methotrexate (MTX). However, it is not known what effect these complications have on MTX clearance when they arise after the plasma MTX concentration has fallen to an undetectable level. We describe the clinical course and pharmacokinetics of MTX in a patient with acute lymphoblastic leukemia who experienced pleural effusions, ascites, and renal failure during the weeks after treatment with high-dose MTX (1.63 g/m^2 i.v. over 24 h). The patient's normal initial MTX clearance rate (107 ml/min/m^2) was consistent with his undetectable plasma level of MTX on day 9 after the infusion. His plasma MTX concentration then gradually increased as his renal function declined, reaching a peak of 0.72 μM on day 15. This unusual finding of an undetectable plasma MTX concentration that subsequently rose to persistent, potentially toxic levels was explained only by a pharmacokinetic model that accounted both for a third space at the time of treatment and for the subsequent decrease in the systemic elimination rate. Therefore, the finding of a physiologic third space during MTX administration combined with the detection of renal dysfunction in the following weeks should be an indication for prolonged therapeutic drug monitoring.

Keywords Methotrexate · Third space · Renal dysfunction · Pleural effusion · Pharmacokinetics

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Introduction

Methotrexate (MTX) is an antimetabolite antineoplastic agent often given by high-dose intravenous infusion. Physiologic third spaces created by pleural effusions, ascites, and gastrointestinal obstruction provide a reservoir for MTX and thereby contribute to delayed excretion [1, 3, 4, 8]. This effect alters MTX kinetics and increases the risk of toxicity.

We describe a patient who was found to have pleural effusions on day 4 after receiving high-dose MTX (HDMTX). His plasma MTX concentration during the first 48 h was consistent with a normal pattern of excretion, and his MTX clearance rate (107 ml/min/m^2) was similar to the population mean (111 ml/min/m^2). Even though his day-9 plasma sample showed no detectable MTX, his MTX plasma concentration steadily rose thereafter as he began to experience acute renal failure.

Case report

The patient was an 18-year-old white male who was diagnosed to have B-cell precursor acute lymphoblastic leukemia (ALL) in July 2001. He presented with a white blood cell count of 3900 μl^{-1} with 24% circulating blast cells, and perianal cellulites which was treated with intravenous meropenem, tobramycin, and vancomycin. Blood and rectal cultures grew *Pseudomonas aeruginosa*.

He attained a complete remission after 6 weeks of induction therapy (consisting of prednisone, vincristine, daunorubicin, asparaginase, cyclophosphamide, mercaptopurine, and cytarabine) without significant toxicity. On attaining complete remission, he received consolidation therapy with intrathecal MTX, hydrocortisone, and cytarabine, followed on the same day by systemic therapy with HDMTX (2.5 g/m^2 i.v. over 24 h) and daily oral mercaptopurine (50 mg/m^2 per day). At hour 42 after the start of the HDMTX infusion, the patient complained of nausea and soreness at the lumbar puncture site. He remained in the hospital overnight for

leucovorin (10 mg/m^2 i.v. every 6 h, for five doses), antiemetics, and i.v. fluid support. The patient was discharged the following day, and his plasma MTX concentration was below 0.1 μM at hour 90. On day 5, grade 2 mucositis was noted. On day 8, the patient complained of pain on both sides of his chest with deep breathing. A diagnosis of pleuritis was made and pain medication was given. On the following day, the patient complained of worsening back pain, which was significantly improved by cyclobenzaprine.

Two weeks after receiving the first course of consolidation therapy, the patient was admitted to the hospital to receive the second course. Per protocol, the MTX dosage was adjusted to 1625 mg/m^2 i.v. over 24 h to achieve a targeted steady-state plasma concentration of 33 μM. His plasma MTX concentration was 21.43 μM at 24 h, and at 42 h it was below the threshold (0.5 μM) for which no additional leucovorin or i.v. fluids were indicated. The patient therefore received five doses of leucovorin (10 mg/m^2 every 6 h) and was discharged. On day 4, the patient was readmitted with a presumptive diagnosis of pleuritic posterior chest pain. The pain was more severe than it had previously been and was not relieved by cyclobenzaprine or oral morphine. A physical examination revealed poor aeration, crackles, and decreased breath sounds at the bases of both lungs. A chest radiograph demonstrated a left pleural effusion.

On day 7, the patient developed fever, hypoxia, tachycardia, and mildly decreased perfusion of his extremities. Oxygen support was required to maintain adequate oxygen saturation. A chest radiograph showed a persistent left pleural effusion, left lower lobe atelectasis, and a small, newly developed right pleural effusion. Although physical examination of the abdomen was normal, a CT scan of the abdomen was consistent with typhlitis (1.9-cm thickness of the descending colon) and pneumatosis intestinalis. Ascites was not documented.

For the next 8 days, his physical conditions remained relatively stable. He continued to require oxygen because of the bilateral pleural effusions. During this time, however, the patient's abdomen became tender to deep palpation and mildly distended. He also became neutropenic during this period. Cultures were negative, and the patient remained on broad antimicrobial coverage.

The plasma MTX concentration was assayed on day 11 after HDMTX because of concern that the pleural effusions diagnosed after HDMTX therapy could have caused delayed excretion of MTX. The MTX concentration was 0.03 μM, which is the lower limit of detection by the fluorescence polarization immunoassay used in our laboratory (Abbott, TDx, Chicago, Ill.) and is therefore considered equivalent to an undetectable concentration. No additional leucovorin was given at that time.

Fourteen days after HDMTX was given, the patient's condition rapidly deteriorated, with an increasing oxygen requirement, respiratory distress, and significantly increased abdominal pain and distension. The serum bilirubin, blood urea nitrogen, and serum creatinine values began to increase. Good urine output was maintained. The patient was transferred to the intensive care unit due to probable septic shock and granulocyte colony-stimulating factor was started. Dopamine therapy was started because of hypotension. A CT scan of the chest documented a new area of nodular density in the right lower lobe and a slightly larger right pleural effusion, which was now loculated. A CT scan of the abdomen revealed increasing inflammatory changes in the peritoneum and retroperitoneum.

In the afternoon of day 15, a therapeutic and diagnostic ultrasound-guided thoracentesis was performed. The MTX concentration was 0.57 μM in the pleural fluid and was unexpectedly found to have risen to 0.62 μM in plasma (Fig. 1). Leucovorin rescue (25 mg/m^2 every 6 h) was initiated and later increased to 100 mg/m^2 every 6 h. Plasma and serum samples that

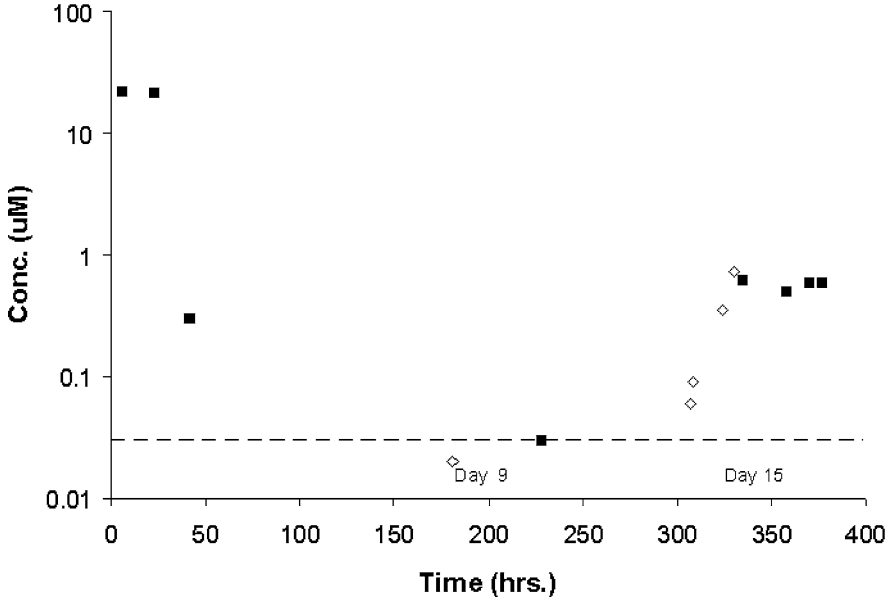

Fig. 1 MTX plasma concentration vs time. This plot includes the results of all available MTX assays, including retrospectively assayed samples (*open diamonds*), from the beginning of the second HDMTX infusion (time 0) to day 17. The *dashed line* shows the limit of quantification of the MTX assay

had been drawn for other clinical tests during the prior few days were assayed and retrospectively demonstrated a gradual rise in the MTX concentration starting on day 9 (Fig. 1). A total parenteral nutrition solution that contained multivitamins (which cause a yellow coloration, as does MTX) was assayed and confirmed to contain no MTX.

The patient continued to deteriorate overnight, with worsening renal and liver function, decreased urine output, respiratory failure, and an increasing need for pressors (now comprising dopamine, epinephrine, and norepinephrine), and ventilatory support. Because of an increased serum lactate value of 17 mmol/l and metabolic acidosis, emergent exploratory laparotomy was performed to rule out bowel necrosis or ischemia. The laparotomy and a diverting ileostomy produced 1 l of clear yellow ascitic fluid, but no bowel perforation was noted.

The patient's renal function continued to decline, and continuous venovenous hemofiltration (CVVH) was started. The patient showed no response to glucocorticoids, white blood cell transfusions, high-dose pressors, and infliximab (a monoclonal antibody targeting tumor necrosis factor α). The patient expired on day 18 after receiving his second course of HDMTX.

An autopsy revealed the cause of death to be septic shock followed by multiple organ failure. Areas of hemorrhage and small infarcts were found throughout the body. There were also multiple caseating granulomas. Post-mortem cultures isolated *Clostridium* and *Bacteroides* species in the cerebrospinal fluid and *Staphylococcus epidermidis* in the ascitic and cerebrospinal fluid. Gram stains showed small budding yeast forms consistent with *Histoplasma* species. The MTX concentration was 0.41 μM in pleural fluid, 0.48 μM in ascitic fluid, and 0.51 μM in ileostomy fluid.

Pharmacokinetic modeling

We pharmacokinetically evaluated this unusual finding of a plasma MTX concentration that first decreased to an undetectable level 9 days after the start of HDMTX (as determined retrospectively) and then increased to a cytotoxic level by day 15. Seven compartmental pharmacokinetic models (Table 1) were constructed and were fitted to the plasma concentration vs time and pleural fluid concentration vs time data by using Bayesian estimation via maximum a posteriori probability (MAP) estimation and maximum likelihood algorithms, as implemented with the ADAPT II software [2].

After remission induction therapy on the St Jude protocol, patients are treated according to the risk of relapse indicated by their presenting features and their level of minimal residual disease after remission induction therapy. For patients assigned to the low-risk group, the MTX dosage is adjusted on the basis of the patient's previous MTX clearance to target a steady-state plasma MTX concentration of 33 μM. Patients receive the conventional dose of 2.5 g/m^2 per 24 h if reliable previous clearance information is not available. The MTX clearance is estimated by using a first-order two-compartment model with the MAP estimation algorithm implemented in ADAPT II. The prior mean (CV%) clearance rate used in the MAP estimation was 101.8 ml/min/m^2 (28%), a value based on MTX pharmacokinetic studies in the prior St Jude protocols [5, 6, 7]. Thus far, the population mean (CV%) clearance, based on plasma samples collected within 42 h, is 111 ml/min/m^2 (26%) for the low-risk group. For the patient, the MTX dose and estimated clearance were 2.5 g/m^2 and 83.6 ml/min/m^2 for the first course and 1.625 g/m^2 and 107 ml/min/m^2 for the second course of consolidation therapy. Therefore the dose of MTX given during the second course was slightly less than the population mean because of the patient's lower-than-average MTX clearance rate during first course. Because the MTX clearance was higher during the second course of consolidation therapy than during the first, the steady-state concentration of MTX achieved during the second course (22 μM) was below the targeted concentration of 33 μM (Fig. 2).

Two-compartment model without time-variant parameters

For this approach it was assumed that the pharmacokinetic parameters estimated during the first 42 h

Table 1 A comparison of the seven pharmacokinetic models used in attempting to describe the patient's plasma MTX concentration vs time data (K_e elimination constant, N/A not applicable)

Model	No of compartments	Two-compartment parameters	Time-variant, K_e	Time-variant third compartment parameters	Predict low concentration day 8?	Predict high concentrations after day 8?
A	2	Estimated on basis of samples $\leq$ 42 h	No	N/A	Yes	No
B	2	Fixed to model A	Yes	N/A	Yes	No
C	2	Estimated on basis of all samples	No	N/A	Yes	No
D	2	Estimated on basis of all samples	Yes	N/A	No	Yes
E	3	Fixed to model A	No	No	Yes	No
F	3	Fixed to model A	Yes	No	Yes	No
G	3	Fixed to model A	Yes	Yes	Yes	Yes

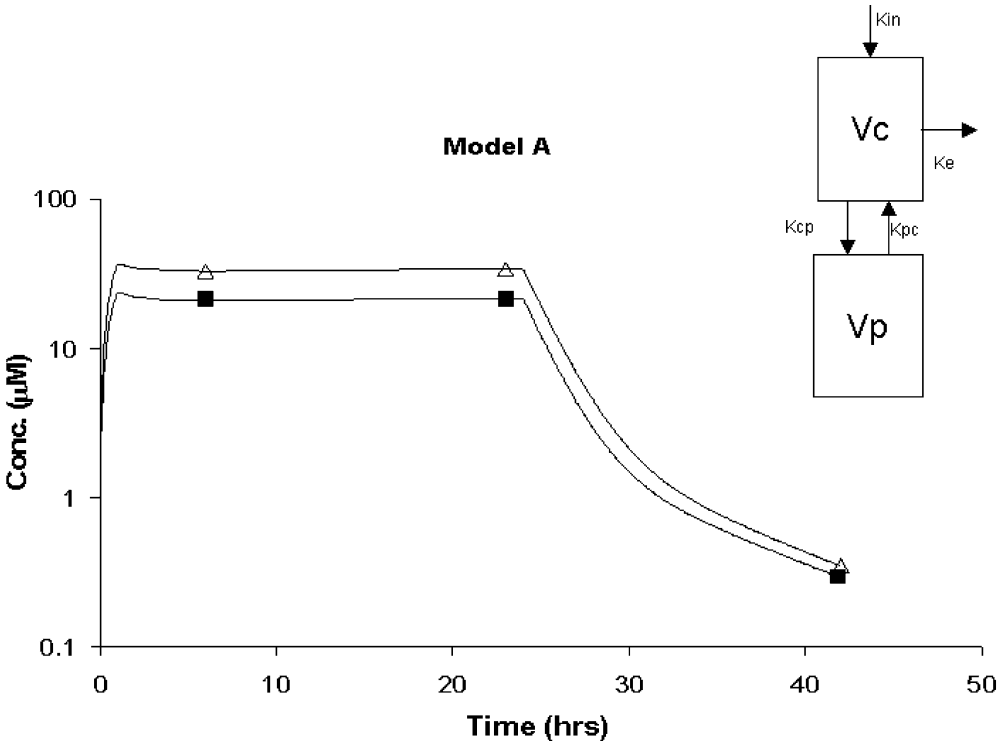

Fig. 2 Comparison of MTX plasma concentration vs time during the first 42 h of the second HDMTX infusion (*open triangles* population mean, *filled squares* patient's values). The population mean MTX clearance was 111 ml/min/m^2 and the patient's MTX clearance rate for this course was 107 ml/min/m^2. The steady-state MTX plasma concentration was 22 μ*M* (patient) and 33 μ*M* (population mean)

remained unchanged for the entire post-HDMTX course (model A). The finding of undetectable MTX on day 9 is consistent with the clearance predicted by this model (Fig. 3). However, the gradual increase in plasma MTX after day 9 is not consistent with either this initial clearance or the standard model.

Two-compartment model with gradual decrease of elimination after day 9

Because renal function had clearly declined between days 9 and 15, we made a simple modification of the two-compartment model to fix the initial pharmacokinetic parameters to those estimated from the patient's data for the first 42 h only, and to allow the elimination rate (K_e) to decrease gradually as a function of increasing serum creatinine concentration after day 9 (model B). The failure of this model to fit the plasma concentrations after day 9 (Fig. 3) demonstrates that the onset of renal dysfunction alone could not account for the unusual observation of increasing MTX concentration.

Two-compartment model with time-variant intercompartmental rate constants

It has been previously demonstrated [3] that the impact of a third space (pleural effusion, gastrointestinal obstruction, or ascites) on delayed plasma MTX excretion can be adequately modeled by allowing the intercompartmental rate constants to reflect altered distribution of MTX from the peripheral second compartment. We tested whether such a model would accommodate the low plasma concentration on day 9 and the higher concentrations between days 11 and 15 (model C). We found that this model could account for the low concentration on day 9 but not for the reappearance of plasma MTX after day 9 (Fig. 3). In model D, in addition to allowing the intercompartmental rate constants to vary, we incorporated a gradual decrease in K_e, caused by a gradual decrement in renal function. Again, this model could not describe the low concentrations on day 9 and the subsequent higher concentrations (Fig. 3).

Three-compartment model without time-variant parameters

To better account for the effects of the third space, model E included an explicit third compartment. Specifically, the parameters related to the original two compartments were fixed to the initial pharmacokinetic parameters estimated from the patient data for the first 42 h. Therefore, only the new third compartment's intercompartmental parameters were fitted (Fig. 4). The failure to fit the later plasma concentrations demonstrated that the onset of renal dysfunction had to be included in the model.

Three-compartment model with gradual decrease of elimination after day 9

Because it was clear that renal function declined between days 9 and 15, a gradual decrease in K_e (based on the

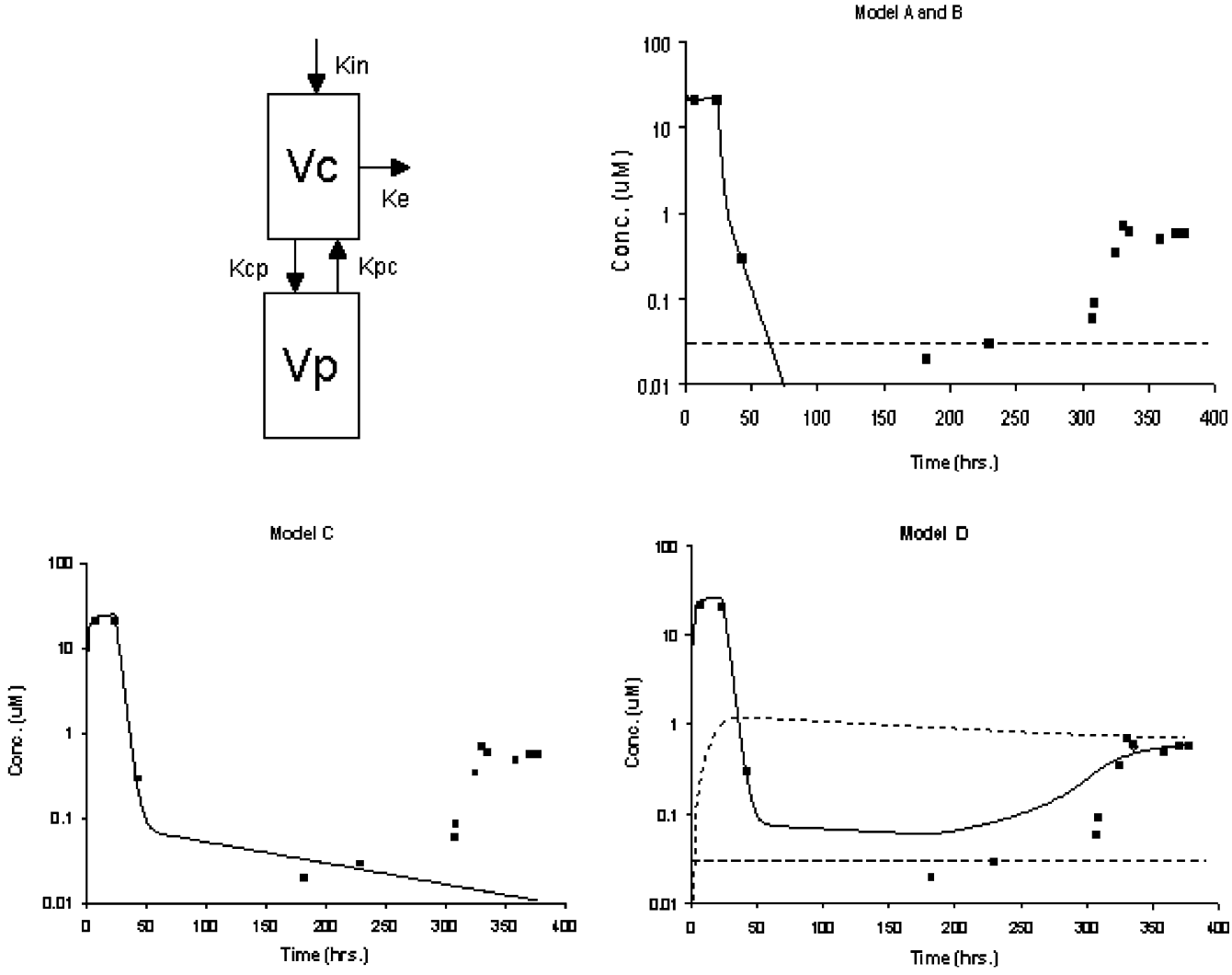

Fig. 3 The two-compartment pharmacokinetic models used to attempt to describe the patient's MTX concentration vs time data. Models A and B fitted to the patient's plasma MTX concentration (*solid curve*); the *dashed line* shows the limit of quantification of the MTX assay. Model C fitted to the patient's plasma MTX concentration (*solid curve*). Model D fitted to the patient's plasma (*filled squares*, *solid curve*) and pleural effusion (*open diamonds*, *dotted curve*) MTX concentration

increasing serum creatinine concentration) was added to model E to create model F. Although much better than the previous models, model F still underestimated the later plasma concentrations (Fig. 4).

Three-compartment model with gradual decrease of elimination after day 9 and time-variant third compartment parameters after day 9

In addition to the assumptions made in the previous model, model G allowed the third compartment intercompartmental parameters to vary around the time of onset of renal dysfunction in a discrete manner, i.e., one set of values before day 10 and another set of values after day 10. This effect is termed a "leaky" third compartment. This modification allowed the model to adequately describe both the low MTX concentration on day 9 and the increased concentration after day 9 (Fig. 4).

Discussion

The patient's undetectable plasma MTX concentration on day 9 is consistent with normal MTX excretion during the first few days after HDMTX administration, and his plasma MTX clearance rate was comparable to the mean rate observed in similarly treated patients. However, the surprising gradual increase in plasma MTX concentration after that point does not fit the standard two-compartment pharmacokinetic model for HDMTX. In retrospect, it appeared that the patient had developed a third space by virtue of fluid accumulation at the time of the second course of HDMTX. The contribution of such third spaces to prolonged excretion of plasma MTX has been well documented and has been related to severe toxicity in several instances [1, 3, 4, 8]. However, if plasma MTX levels are monitored adequately for a

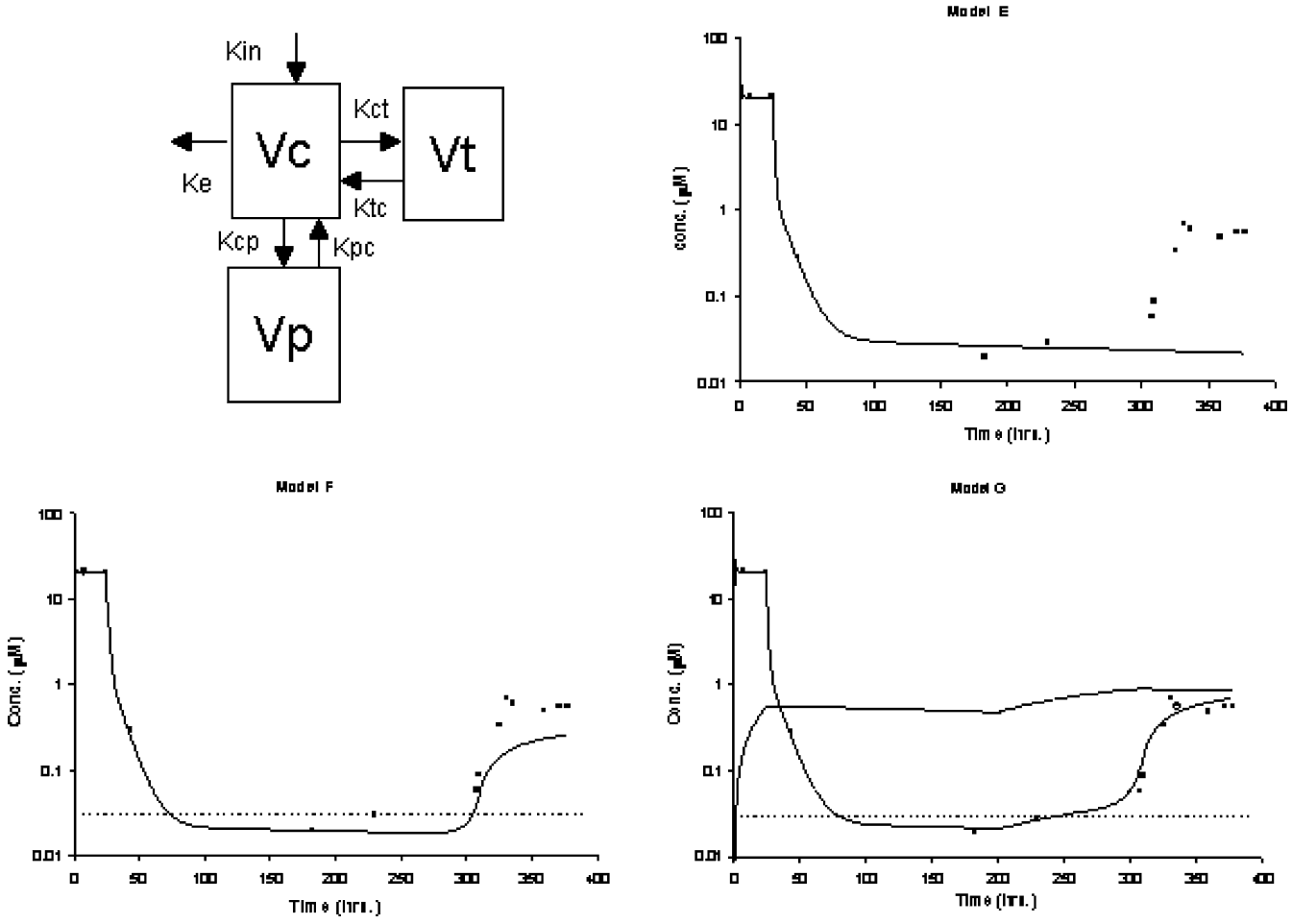

Fig. 4 The three-compartment pharmacokinetic models used to attempt to describe the patient's MTX concentration vs time data. The *dashed line* represents the limit of quantification of the MTX assay. **a** Model E fit to the patient's plasma MTX concentration (*solid curve*). **b** Model F fit to the patient's plasma MTX concentration (*solid curve*). **c** Model G fit to the patient's plasma (*filled square*, *solid curve*) and pleural effusion (*open diamond*, *dotted curve*) MTX concentration

prolonged period, the effect of a third space on MTX excretion and toxicity can be ameliorated by adjusting leucovorin rescue on the basis of the plasma MTX value. In this case, because the plasma MTX concentration was below the threshold indicator for continued monitoring at 42 h and no third space was suspected, there was no reason initially to attribute the observed effects to MTX toxicity; therefore, plasma MTX concentrations were not monitored between days 3 and 9.

After pleural effusions were documented and toxicity was observed, the plasma MTX concentration was at the lower threshold of detection (0.03 μM). When renal dysfunction subsequently developed, it was not suspected to be a contributing factor in any MTX-induced toxicity, because the last HDMTX infusion had been given 2 weeks previously and the plasma MTX concentration had declined to a level considered to be negligible. Surprisingly, we found that the plasma MTX concentration had increased to a potentially toxic value (>0.5 μM) more than 2 weeks after the HDTMX infusion. Therefore, we considered the possibility that MTX in a third space had contributed to the increasing plasma MTX concentration with the onset of renal failure. The presence of a third space escaped diagnosis at the time of therapy and did not contribute to elevated plasma MTX concentration while renal function was adequate. Furthermore, the possibility was implausible enough that we actually tested the patient's intravenous parenteral nutrition fluid to rule out inadvertent administration of MTX.

Using pharmacokinetic modeling in a systematic fashion, we ruled out the possibility that either the onset of a third space alone or the onset of late renal dysfunction alone could have resulted in the unusual pattern of plasma MTX concentrations observed in this patient. In fact, both factors—development of a third space on the day of HDMTX or the following day plus the onset of delayed renal dysfunction—were required to adequately describe the time-course of plasma and pleural fluid MTX concentration. In retrospect, it is certainly possible that MTX exposure in the absence of leucovorin rescue between days 9 and 15 contributed to the clinical deterioration of this patient in the weeks after administration of HDMTX.

To assess the prevalence of "delayed" renal dysfunction following HDMTX, we searched the computer records of St Jude Children's Research Hospital from 1992 to 2001 for all HDMTX courses with episodes of elevated serum creatinine values (>0.8 mg/dl in patients less than 1 year of age, >1.4 mg/dl in patients 1–10 years of age, >2.0 mg/dl in patients greater than 10 years of age) which occurred within 30 days of the HDMTX treatment. Among 6861 courses, 33 were associated with delayed renal dysfunction. Of those 33 courses, 8 also had elevated MTX concentrations (i.e., the 44-h MTX level was greater than 1.0 μM) and thus were closely monitored until MTX plasma concentrations were below toxic thresholds. Therefore, 25 of 6861 courses (0.36%) without delayed MTX excretion had renal dysfunction within the following 4 weeks. Although it appears to be a very rare occurrence, the onset of renal dysfunction in the weeks following HDMTX, even in a patient who initially has normal clearance, may be an indication for continued and aggressive therapeutic drug monitoring. If the MTX plasma concentration is elevated, a search for a third space should be made and leucovorin rescue reinstituted.

Our practice has been to discontinue therapeutic drug monitoring of patients receiving HDMTX when their plasma MTX concentration reaches <0.5 μM 42–48 h after the infusion, unless there are concurrent risk factors for delayed excretion (poor renal function, pleural effusion, ascites, or gastrointestinal obstruction). In the latter cases, our practice has been to monitor plasma MTX concentration until it reaches <0.1 μM. In the present case, it is not known whether the plasma MTX concentration would have remained >0.1 μM for an unusually long period in the days immediately after the MTX infusion, but the pharmacokinetic modeling suggests that this would not have been the case. Therefore, continued therapeutic drug monitoring during that early period might not have made a difference to the outcome. However, had we continued daily monitoring of MTX after renal function deteriorated, we would have observed the gradual increase in plasma MTX and would have been able to reinstitute leucovorin rescue earlier. Therefore, we now monitor plasma MTX for several days after giving HDMTX in all cases with suspected third space or renal dysfunction, and we reinstitute leucovorin therapy if the plasma MTX concentration becomes detectable.

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