Carboxypeptidase G₂ rescue in patients with methotrexate intoxication and renal failure

S Buchen¹, D Ngampolo¹, RG Melton², C Hasan¹, A Zoubek³, G Henze⁴, U Bode¹ and G Fleischhack*¹

¹Department of Paediatric Haematology/Oncology, Children's Medical Hospital, University of Bonn, Bonn, Germany; ²Toxigenics Plc, Salisbury, UK; ³St. Anna Children's Hospital, Vienna, Austria; ⁴Department of Paediatric Haematology/Oncology, Charité, Humboldt University of Berlin, Germany

The methotrexate (MTX) rescue agent carboxypeptidase G₂ (CPDG₂) rapidly hydrolysates MTX to the inactive metabolite DAMPA (4-(2,4-diamino-6-(pteridinyl)methylamino)-benzoic acid) and glutamate in patients with MTX-induced renal failure and delayed MTX excretion. DAMPA is thought to be an inactive metabolite of MTX because it is not an effective inhibitor of the MTX target enzyme dihydrofolate reductase. DAMPA is eliminated more rapidly than MTX in these patients, which suggests a nonrenal route of elimination. In a phase II study (May 1997–March 2002), CPDG₂ was administered intravenously to 82 patients at a median dose of 50 U kg⁻¹ (range 33–60 U kg⁻¹). Eligible patients for this study had serum MTX concentrations of >10 μM at 36 h or >5 μM at 42 h after start of MTX infusion and documented renal failure (serum creatinine ≥1.5 times the upper limit of normal). Immediately before CPDG₂ administration, a median MTX serum level of 11.93 μM (range 0.52–901 μM) was documented. Carboxypeptidase G₂ was given at a median of 52 h (range 25–178 h) following the start of an MTX infusion of 1–12 g m⁻³ for 36 h and resulted in a rapid 97% (range 73–99%) reduction of the MTX serum level. Toxicity related to CPDG₂ was not observed. Toxicity related to MTX was documented in about half the patients; four patients died despite CPDG₂ administration due to severe myelosuppression and septic complications. In conclusion, administration of CPDG₂ is a well-tolerated, safe and a very effective way of MTX elimination in delayed excretion due to renal failure.

British Journal of Cancer (2005) 92, 480–487. doi:10.1038/sj.bjc.6602337 www.bjcancer.com

Keywords: high-dose methotrexate; acute renal failure; methotrexate toxicity; carboxypeptidase G₂
successfully to a small number of patients with brain tumours and to one patient with pre-existing renal failure after MTX administration. However, the bacterial source for CPDG2 is no longer available (Bertino et al, 1974; Abelson et al, 1978; Minton et al, 1983).

A recombinant form of CPDG2, cloned from Pseudomonas strain RS-16, is now available as an investigational drug (Minton et al, 1983; Sherwood et al, 1985). Carboxypeptidase G2 hydrolyses MTX rapidly to the inactive metabolites DAMPA (4-[2,4-diamino-6-(pteridinyl)methyl]-methylamino]-benzoic acid) and glutamate. In contrast to CPDG1, CPDG2 has a higher affinity for MTX (Km 8 μM) than LV (Km 120 μM) and 5-methyltetrahydrofolate (Km 35 μM), the primary circulating metabolite of LV. In studies performed in rheumatoid arthritis, a single CPDG2 dose of 50 units (U) kg\(^{-1}\) lowered serum MTX steady-state concentration from 10 μM to nontoxic levels of <0.05 μM within 30 min (Adamson et al, 1992). In addition, the bolus injection of CPDG2 (a dose of 50 U kg\(^{-1}\) in combination with LV and partial thymidine rescue) resulted in a rapid 95–99% reduction of plasma levels of MTX in patients with renal dysfunction after HD-MTX (Widemann et al, 1995, 1997; Zoubek et al, 1995).

This study will demonstrate the feasibility, effectiveness and toxicity of an emergency therapy with CPDG2 in a large European patient collective with acute MTX intoxication and renal failure.

**PATIENTS AND METHODS**

**Patients**

**Study design** The study was a prospective, open, nonrandomised, multicentre trial. The primary study centres were in Bonn, Berlin and Vienna. Their task consisted of consultation and emergency dispatch of CPDG2 for patients with MTX intoxication. The performance was requested by 50 different hospitals in 13 different countries (Austria, Belgium, Czechia, France, Germany, Greece, Italy, Israel, Norway, Slovakia, Spain, Sweden and Switzerland).

**Patient eligibility** Patients of any age were eligible if they had (1) serum MTX concentration of >10 μM at 36 h or of >5 μM at 42 h or of >3 μM at 48 h after start of MTX infusion, and (2) decreased diuresis (less than 50% excretion of the input hydration) or (3) a serum creatinine (Cr\(_{\text{e}}\)) >1.5 times the upper limit of normal and documented increase of Cr\(_{\text{e}}\) during the infusion period.

**Treatment**

**Carboxypeptidase G2 administration** Recombinant CPDG2 was manufactured by the Centre for Applied Microbiology and Research (Salisbury, UK) and supplied in lyophilised form with vials containing 1000 U of enzyme activity. The vials were each resuspended in 1 ml of sterile isotonic saline; further dilution with sterile isotonic saline (1:5 or 1:10) was recommended. Carboxypeptidase G2 was administered at a dose of 50 U kg\(^{-1}\) over 5 min intravenously by an infusion pump or by bolus injection. Following each dose of CPDG2, serum MTX concentrations were determined. Patients who experienced a decrease in serum MTX concentration greater than one logarithm following CPDG2 administration but still had serum MTX concentrations >1 μM might receive additional doses of CPDG2 with the approval of the principal investigator of the study.

**Leucovorin** Leucovorin administration had to be stopped 4 h prior to CPDG2 and readministered 1 h following enzyme injection. The recommended LV dose during the first 24 h after the CPDG2 injection was 100 mg m\(^{-2}\) every 6 h followed by an increased rescue for 5 days according to the scheme recommended in the ALL BFM protocol (Schrappe et al, 1995).

**Patient monitoring** Patients were evaluated daily for signs and symptoms of MTX toxicity. Complete blood counts with differential, bilirubin and ALT, AST were determined at least twice weekly. Recovery of renal function was documented daily by serum creatinine. The nonhaematological (i.e. renal, liver, neurological, gastrointestinal toxicity and infectious complications) and haematological toxicity of MTX and side effects of CPDG2 (especially allergic reactions) were determined according to National Cancer Institute Common Toxicity Criteria (CTC) version 2.0.

**Sampling for determination of MTX concentration** EDTA samples for MTX determination were obtained immediately before and 15, 30, 60 and 120 min following the dose of CPDG2, and then once daily until recovery of kidney function and decrease of MTX serum levels to less than 0.1 μM. Most of the local hospitals determined the MTX level immediately with the methods such as fluorescence polarisation immunoassay (TDX) or enzyme-multiplied immuno-technique (EMIT). In a few centres, additional blood samples for analysis by high-pressure liquid chromatography (HPLC) were placed on ice, and serum was rapidly separated by centrifugation. To inactivate CPDG2, the serum samples were heated to greater than 80°C for 5 min in a water bath or treated with 1 N HCl to give a final concentration of 0.1 N hydrochloric acid. If the enzyme activity in the samples was not fully stopped, MTX in the sample might continue to be degraded after collection. Serum was stored at −20°C until time of analysis. All serum samples were sent on dry ice to a central laboratory where they were stored at −20°C until analysis by HPLC.

**Methods**

**DAMPA, the cabatolic product of CPDG2 action on MTX, is known to crossreact with MTX in most commercial immunological MTX assays (Allegra, 1990; Albertioni et al, 1996; Widemann et al, 1997). Consequently, MTX concentrations determined by commercial laboratories are unreliable following treatment with CPDG2. Methotrexate must be assayed by a specific HPLC or enzyme inhibition method.**

**High-pressure liquid chromatography method** Serum MTX was measured by HPLC using a modification of a previously reported method (Brandstetterova et al, 1990). After solid phase extraction using C18 cartridges (WatersOasis HLB 3 cm\(^{3}\), 60 mg (Waters, Eschborn, Germany)), samples were injected onto a 3 mm, Supelcosil LC-18-DB 15 cm × 4.6 mm (Supelco, München, Germany) radial compression analytical column with a Supelguard LC-18-DB 5 mm (Supelco, München, Germany) guard column and eluted isocratically with 80:20 (v:v) 0.1 mol l\(^{-1}\) sodium phosphate (pH 6.8): methanol (Lichrosolv\(^{R}\)) at a flow rate of 1.2 ml min\(^{-1}\). Eluent was monitored using a Waters 490 UV adjustable wavelength detector at a wavelength of 310 nm (ThermoQuest, Egelsbach, Germany).

Under these conditions, the retention times for MTX, DAMPA and the internal standard 4-[2,4-diamino-6-(pteridinyl)methyl]amino]-benzoic acid (Dm-APA) were approximately 5.6, 9.5 and 3.5 min, respectively. The peak areas were evaluated using the Chromquest software (2.5.1, 1998, ThermoQuest, Egelsbach, Germany). Methotrexate and DAMPA were corrected by means of an internal standard in combination with a calibration curve.

**Statistical analysis**

Patient data were collected consecutively, recorded on data sheets prepared centrally beforehand, checked by the principal investigator and recorded in a protected database at the University of Bonn. The biometric analysis was performed for all eligible patients who were evaluable (1) for response to the study...
medication, (2) for the toxicity of CPDG2 and (3) for the toxicity of MTX due to complete data documentation. Data were evaluated using descriptive statistical methods (median, ranges, frequencies and percentages).

Ethics

The study was conducted in accordance with the updated declaration of Helsinki (1996, Somerset West, Republic of South Africa) and approved by the local ethics committees in Bonn, Berlin and Vienna. Prior to enrolment in the study, the patient’s parents/legal representatives and/or the patients themselves were informed of the investigational character of the study and the emergency use of CPDG2 and had given their written informed consent.

RESULTS

Patient characteristics

A total of 82 patients from institutions in Europe were enrolled in this compassionate and emergency use protocol between May 1997 and March 2002. Complete or nearly complete data were available from 65 patients. For 17 patients, no data are available despite repeated telephone and written efforts. The evaluable 65 patients aged 0.9–71.8 years (median 15.4 years) suffered from acute lymphoblastic leukaemia (n=26), non-Hodgkin’s lymphoma (NHL; n=21), osteosarcoma (n=12), brain tumours (n=3), Hodgkin’s lymphoma (n=2) and pleural mesothelioma (n=1). The patient’s characteristics and the different MTX regimens are listed in Table 1. Except for one patient who had clearly increased serum creatinine of 11.93 μmol l⁻¹, all patients had an age-dependent normal value of CreaS before treatment (range 17.7–120.4 μmol l⁻¹, median 60.2 μmol l⁻¹). All patients received intravenous hydration and alkalinisation before, during and after the MTX infusion.

Contact

Contact with the study centres was established by telephone at a median of 47.5 h (range 19–142 h) after start of the MTX infusion. The MTX levels at the time of contact were between 0.52 and 901 μg l⁻¹ (median 18.0 μg l⁻¹) (n=65). Two patients were included in the study with MTX levels of 0.52 μg l⁻¹ at 94 h (case 1) and 1 μg l⁻¹ at 142 h (case 2) after start of MTX and a serum creatinine of six-fold of the initial value. A total of 12 patients had no documented increase of serum creatinine at the time of contact in contrast to the inclusion criteria. Despite the absent renal failure, the principal investigator (UB) decided to give CPDG2 due to very high MTX levels at a late time of contact.

Methotrexate levels and serum creatinine at the first contact and before CPDG2 administration are documented in Table 1. Except for one patient who had clearly increased MTX levels at a late time of contact.

Table 1: Patient characteristics and MTX serum level at the time of contact.

| Patient characteristics | Serum creatinine (μmol l⁻¹) |
|-------------------------|-----------------------------|
| Underlying disease      |                            |
| ALL                     |                            |
| Number of cases         | 26                          |
| Median age (years)      | 14.1 (24.4–71.8)            |
| Sex (male/female)       | 18/8                        |
| Median MTX dose (g m⁻¹) | 4 (1–12)                    |
| Median serum creatinine | 99 (35.4–220.4)             |
| Median MTX level (μg l⁻¹) | 10.66 (0.52–228)         |
| Median serum creatinine | 52 (25–178)                |
| Median MTX level (μg l⁻¹) | 45.1 (17.7–115.1)         |
| Median serum creatinine | 70.8 (35–178)              |
| Median MTX level (μg l⁻¹) | 136.3 (53.1–516.8)        |

| NHL/Hodgkin’s lymphoma  |                            |
| Number of cases         | 12                          |
| Median age (years)      | 53.3 (4.3–68.9)             |
| Sex (male/female)       | 9/3                         |
| Median MTX dose (g m⁻¹) | 3 (1–8)                     |
| Median serum creatinine | 7.46 (0.8–260)              |
| Median MTX level (μg l⁻¹) | 45.1 (4.4–144.3)          |
| Median serum creatinine | 7.48 (0.8–260)             |
| Median MTX level (μg l⁻¹) | 136.3 (53.1–516.8)        |

| Osteosarcoma            |                            |
| Number of cases         | 12                          |
| Median age (years)      | 15.3 (9.3–39.4)             |
| Sex (male/female)       | 9/3                         |
| Median MTX dose (g m⁻¹) | 12                          |
| Median serum creatinine | 63.7 (44.3–97.4)            |
| Median MTX level (μg l⁻¹) | 136.3 (53.1–516.8)        |
| Median serum creatinine | 64.5 (44.3–97.4)            |
| Median MTX level (μg l⁻¹) | 136.3 (53.1–516.8)        |

| Brain tumour/pleural mesothelioma |                            |
| Number of cases | 31                          |
| Median age (years) | 3 (1–11)                    |
| Sex (male/female) | 21/10                        |
| Median MTX dose (g m⁻¹) | 12                          |
| Median serum creatinine | 63.7 (44.3–97.4)            |
| Median MTX level (μg l⁻¹) | 136.3 (53.1–516.8)        |
| Median serum creatinine | 64.5 (44.3–97.4)            |
| Median MTX level (μg l⁻¹) | 136.3 (53.1–516.8)        |

Carboxypeptidase G2 administration

Immediately before CPDG2 administration, a median MTX serum level of 11.93 μmol l⁻¹ (range 0.52–901 μmol l⁻¹) was documented (n=58).

A single dose of 50 U CPDG2 per kg body weight was intended. A vial CPDG2 contains 1000 U dry frozen. In all, 57 of 58 evaluable patients received a dose of between 33 and 60 U CPDG2 per kg body weight (median 50 U kg⁻¹). One patient received only a dose of 17 U CPDG2 kg⁻¹, although the correct amount was available. Carboxypeptidase G2 was administered at a median of 52 h (range 25–178 h) following the start of an MTX infusion of 1.5–12 g m⁻² over 4–36 h and was well tolerated. At 15 min after CPDG2 administration, the MTX serum level of these patients was reduced by around 87%.
Nine patients received a second dose of CPDG₂. One patient received an additional third dose of CPDG₂. Six of these nine patients suffered from an osteosarcoma and received an MTX therapy of 12 g m⁻² 24h⁻¹ (n = 2) or 8 g m⁻² 6h⁻¹ (n = 1), respectively. The median time point of the first CPDG₂ dose in these patients was 37.5 h after the start of MTX infusions. The second dose was given at a median of 6 h (range 1.5–90 h) after the first dose. One patient received a second dose of CPDG₂ 1.5 h after the first dose of CPDG₂ due to logistics.

Side effects of CPDG₂

After the administration of CPDG₂, side effects described by two patients included flushing (n = 2) and shaking (n = 1). Both had stable vital signs and the symptoms resolved completely without any intervention.

Methotrexate levels

Carboxypeptidase G₂ resulted in a rapid reduction of serum MTX concentrations in all patients. The MTX concentration determined with immunological MTX detection such as TDX or EMIT showed a median decrease of the MTX level of about 87% (range 70–99%) 15 min after CPDG₂ administration. Subsequent samples also showed an ongoing decrease of MTX concentrations. Additionally, the MTX concentrations after CPDG₂ administration were determined by the HPLC method. The MTX levels estimated by HPLC 15 min after the first CPDG₂ injection decreased by a median of 97% (range 73–99%) (Figure 1). Almost immediately after CPDG₂ administration, DAMPA was detectable at a concentration comparable to the serum concentration of MTX before CPDG₂.

Methotrexate-related toxicity (CTC)

In all, 50% of the patients had a reduced Karnowsky index (<60%) due to the MTX intoxication. Gastrointestinal complaints with nausea and diarrhoea were frequent. Severe stomatitis (CTC scale ≥2) occurred in 33% of the patients. A total of 66% of the patients showed an increase of serum creatinine of more than 1.5 times of the initial value before MTX therapy. Increased liver values based on increased GPT/GOT were documented in 58% of the patients with CTC scale ≥2. Only a small number of patients suffered high-grade toxicity of lung and heart/circulation. In five and six patients, respectively, a mild peripheral and/or central neurotoxicity was observed. Two patients suffered from severe central neurotoxic side effects, a female patient additionally from severe toxine.

---

**Figure 1** Number and time of MTX samples determined by TDX/EMIT or HPLC and DAMPA samples determined by HPLC after CPDG₂ injection.
peripheral neurotoxic side effects (CTC scale 4). Further data are listed in Table 3.

### Methotrexate-related mortality

Four patients died due to severe myelosuppression and septic complications 7–22 days following the MTX infusion. Three patients (Table 4, cases 1, 2 and 4) had very high MTX concentrations several hours after the start of MTX, which were lowered by CPDG 2 insufficiently. Thus, progressive renal failure and fatal septic complications were observed. In an additional patient (case 3), the MTX concentration was lowered by CPDG 2 adequately without progressive renal failure. This patient died due to prolonged myelosuppression and its complications (pulmonary haemorrhage due to pulmonary aspergillosis), which were caused by the MTX intoxication and the progressive underlying disease (T-cell ALL) too.

The patient characteristics are shown in Table 4. The course of MTX serum levels is documented in Figure 2.

### Discussion

Methotrexate-induced toxicity is frequently associated with delayed MTX excretion due to renal dysfunction. Delayed MTX excretion leads to prolonged drug exposure and the potential for severe life-threatening toxicity. Patients with impaired renal function prior to the MTX infusion, of advanced age or receiving concomitant nonsteroidal anti-inflammatory drugs or other nephrotoxic substances are at an increased risk of developing renal dysfunction during MTX infusion. In this study, a pre-existing reduced kidney function was known before initiating the MTX therapy in only one patient. All other patients showed increased MTX concentrations unexpectedly without risk factors.

---

**Table 2** Characteristics of patients who got a second CPDG 2 injection

| Underlying disease | Age (years) | MTX level prior to first CPDG 2 (μM) | Dose of first CPDG 2 (U kg⁻¹) | Time after MTX start (h) | MTX decrease (%) | MTX level prior to-second CPDG 2 (μM) | Dose of second CPDG 2 (U kg⁻¹) | Time after MTX start (h) | MTX decrease (%) | Last MTX level (μM) |
|--------------------|-------------|-------------------------------|-------------------------------|--------------------------|-----------------|-----------------------------|-------------------------------|--------------------------|-----------------|------------------|
| ALL                | 17.1        | 29.7                          | 33                            | 58                       | 77              | 7.6                         | 33                            | 64                       | 26.32           | 0.21             |
| NHL                | 10.2ᵃ        | 45                            | 45                            | 143.5                    | 91              | 3.1                         | 22                            | 192                      | 69.86           | 3.4              |
| NHL                | 55           | 62                            | 47                            | 34.2                     | 84              | 9.7                         | 47                            | 48.2                     | 8.25             | 0.07             |
| Histiocytoma       | 39.4ᵃ        | 688                           | 54                            | 37.5                     | 88              | NA                          | 54                            | 39                       | NA              | 8.06             |
| Osteosarcoma       | 15.4         | 306                           | 46                            | 63.4                     | 74              | 13.4                        | 46                            | 84.4 (⁻)                 | 124.5           | 43.82            |
| Osteosarcoma       | 27.9         | 138                           | 36                            | 34.5                     | 94              | 8.9                         | 53                            | 124.5                     | 43.82           | 0.1              |
| Osteosarcoma       | 9.4          | 596                           | 50                            | 32                       | 84              | 77.6                        | 20                            | 38                       | 4.64             | 0.83             |
| Osteosarcoma       | 19.1ᵃ        | 815                           | 47                            | 24.8                     | 89              | 87.5                        | 47                            | 28.5 (⁻)                 | 2.29ᵇ           | 25.49            |
| Osteosarcoma       | 14.4         | 901                           | 45                            | 50.8                     | 90              | 1.26                        | 45                            | 56                       | NA              | NA              |

ᵃPatient died.ᵇNegative values are due to a rise of the MTX concentration. NA = not available.

---

**Table 3** Methotrexate-related toxicity

| Kind of toxicity | Number of evaluable patients (n) | CTC scale (number of patients (n) (%)) |
|------------------|----------------------------------|--------------------------------------|
|                  |                                  | 0        | 1        | 2        | 3        | 4        |
| Infection        | 40                               | 27 (67.5)| 1 (2.5)  | 7 (17.5) | 1 (2.5)  | 4 (10.0) |
| Fever            | 38                               | 26 (68.4)| 3 (7.9)  | 8 (21.1) | 1 (2.6)  | 0 (0)    |
| Gastrointestinal |                                  | 13 (30.2)| 11 (25.6)| 8 (18.6) | 7 (16.3) | 4 (9.3)  |
| Nausea           | 39                               | 12 (30.8)| 11 (28.2)| 7 (17.9) | 7 (17.9) | 2 (5.1)  |
| Oral mucositis   | 39                               | 17 (43.6)| 9 (23.1) | 7 (17.9) | 6 (15.4) | 0 (0)    |
| Diarrhoea        | 41                               | 24 (58.5)| 9 (22.0) | 5 (12.2) | 1 (2.4)  | 2 (4.9)  |
| Skin             | 42                               | 29 (69.0)| 9 (21.4) | 4 (9.5)  | 0 (0)    | 0 (0)    |
| Haemorrhage      | 39                               | 32 (82.1)| 2 (5.1)  | 2 (5.1)  | 0 (0)    | 3 (7.7)  |
| Kidney           | 41                               | 7 (17.1) | 7 (17.1) | 13 (31.7)| 9 (22.0) | 5 (12.2) |
| Creatinine       | 25                               | 6 (24.0) | 5 (20.0) | 4 (16.0) | 5 (20.0) | 0 (0)    |
| Creatinine clearance | 31                          | 21 (67.7)| 5 (16.1) | 3 (9.7)  | 1 (3.2)  | 1 (3.2)  |
| Proteinuria      | 34                               | 23 (67.6)| 7 (20.6) | 3 (8.8)  | 0 (0)    | 1 (2.9)  |
| Haematuria       | 40                               | 12 (30.0)| 5 (12.5) | 10 (25.0)| 6 (15.0) | 7 (17.5) |
| Liver            | 40                               | 20 (50)  | 12 (0)   | 7 (17.5) | 0 (0)    | 1 (2.5)  |
| GOT/GPT          | 40                               | 24 (60.7)| 12 (0)   | 7 (17.5) | 0 (0)    | 1 (2.5)  |
| Bilirubin        | 40                               | 33 (82.5)| 0 (0)    | 1 (2.5)  | 3 (7.5)  | 3 (7.5)  |
| Liver clinically | 27                               | 36 (90.0)| 0 (0)    | 1 (2.5)  | 0 (0)    | 3 (7.5)  |
| Haematuria       | 19                               | 18 (94.7)| 0 (0)    | 0 (0)    | 0 (0)    | 1 (5.3)  |
| Lung             | 38                               | 31 (81.6)| 5 (13.2)| 0 (0)    | 0 (0)    | 2 (5.3)  |
| Heart/circulation| 38                               | 31 (81.6)| 6 (15.8)| 0 (0)    | 0 (0)    | 1 (2.6)  |

British Journal of Cancer (2005) 92(3), 480 – 487 © 2005 Cancer Research UK
Rescue with LV can prevent toxicity but has no effect on delayed MTX excretion. Carboxypeptidase G2 rescue offers the distinct advantage of providing an alternative, rapid route of elimination of MTX by enzymatically catabolising the drug to the inactive metabolite DAMPA. DAMPA is a minimal inhibitor of dihydrofolate reductase and less soluble than MTX in acidic pH. The faster elimination of DAMPA by comparison with MTX in patients with renal failure, who received CPDG2, is due to the extrarenal elimination of DAMPA (Widemann et al, 2000). After systemic exposure to

Table 4 Characteristics of patients who died related to MTX intoxication

| Case | 1 | 2 | 3 | 4 |
|------|---|---|---|---|
| Diagnosis | Histiocytoma | NHL | ALL | Osteosarcoma |
| Age (years) | 39.4 | 10.2 | 5.7 | 19.1 |
| Time of death (days after MTX start) | 7 | 14 | 21 | 22 |
| Causes of death | Septic, multiorgan failure | Septic, multiorgan failure | Septic, multiorgan failure, progressive underlying disease, haemorrhage due to pulmonary aspergillosis | Cardiopulmonary failure due to severe anaemia, being Jehovah witness |
| MTX therapy | 12 g m⁻² 4 h⁻¹ | 5 g m⁻² 24 h⁻¹ | 1 g m⁻² 36 h⁻¹ | 12 g m⁻² 4 h⁻¹ |
| Contact after MXT start (h) | 32 | 140 | 49.5 | 19 |
| MTX level prior to first CPDG2 (µg/l) | 688 | 9.2 | 4.58 | 814.6 |
| Creatinine concentration prior to first CPDG2 administration (µmol l⁻¹) | 194.7 | 150.5 | 69 | 327.5 |
| Maximum creatinine level (µmol l⁻¹) | 230.1 | 416 | 85.8 | 567.3 |
| Dose of first CPDG2 (U kg⁻¹) | 54 | 45 | 50 | 47 |
| Time after MTX start (h) | 37.5 | 143.5 | 50 | 25 |
| MTX level (µg/l)/MTX decrease (%) after CPDG2 | 82.8/88 | 0.85/91 | 1.46/86 | 86.1/90 |
| MTX level prior to second CPDG2 (µg/l) | NA | 3.1 | 86.1 |
| Dose of second CPDG2 (U kg⁻¹) | 54 | 22 | 47 |
| Time of second CPDG2 after MTX start (h) | 39 | 192 | 28.5 |
| MTX level (µg/l)/MTX decrease (%) after second CPDG2 | 0.94/70 | 87.5/1.6 |
| Leucovorin rescue | Adequately | Not known | Adequately | Adequately |
| Haemodialyse/haemofiltration (days) | 3 | 8 | 5 |
| Start of haemodialysis after MTX infusion (h) | 55 | 171 | 24 |
| Severe myelosuppresion | No | Yes | Yes | Yes |

*Negative value is due to a rerise of the MTX concentration. NA = not available.

Figure 2 Methotrexate levels after first and second CPDG2 injection in patients who died related to MTX intoxication.
CPDG₂, DAMPA serum concentrations are equivalent to MTX concentrations prior to CPDG₂ (compare Figure 1). Because of the risk of additional nephrotoxicity of DAMPA, patients must continue to receive fluid hydration and alkalisation.

- After the initial dose of CPDG₂, all patients experienced a rapid (<1h) and marked (>97%) decrease in serum MTX concentration (determined by HPLC).

In the first years of this study, nine patients received a second dose of CPDG₂ not resulting in a further marked decrease of the MTX concentration. This could be the result of high DAMPA concentrations inhibiting the hydrolysis of MTX by CPDG₂ (Widemann et al., 1997).

In addition, substantial crossreactivity of the MTX antibodies with DAMPA used in immunoassays results in overestimation of serum MTX concentrations. Therefore, the MTX and DAMPA serum concentration after CPDG₂ must be monitored by another technique, such as HPLC. The MTX levels estimated by HPLC decreased in median to less than 3% (range 1-27%) of the initial values 15 min after CPDG₂ injection.

Due to its molecular size, CPDG₂ is restricted to the extracellular compartment, and the intracellular MTX concentration is initially unaffected by its use. In time, the changed equilibrium between intracellular and extracellular MTX will result in the efflux of intracellular MTX back into the serum, resulting in a rise of serum MTX levels some hours after CPDG₂ administration, but this is a relatively slow process. For this reason, rescue with LV must be continued following the CPDG₂ administration.

In this study, six of 65 patients were treated additionally by haemofiltration or haemodialysis. These methods must be repeated continuously or daily, in order to reduce the MTX level to nontoxic concentrations. A further risk of these methods results from the necessary vascular entrance, as well as the risk of bleeding secondary to heparinisation and thrombocytopenia. The effectiveness of these procedures cannot be evaluated from our data. Three patients deceased despite these approaches.

In total, four patients died despite CPDG₂ injection due to severe myelosuppression and septic complications 7-22 days following the MTX infusion. The probable reasons for the lethal complications during a prolonged myelosuppression were (1) very high MTX level with an inadequate decrease of MTX levels after the second CPDG₂ injection, (2) delayed CPDG₂ injection (143.5 h after the start of MTX infusion), (3) a progressive underlying disease (T-cell ALL) and (4) inadequate treatment of severe anaemia in a patient who was a Jehovah witness and refused treatment.

In summary, systemic administration of CPDG₂ followed by long-term LV is highly effective in rescuing patients at high risk of life-threatening MTX toxicity. Carboxypeptidase G₂ is safe and well tolerated. No severe side effects were observed in this study.

Administration of CPDG₂ is most beneficial for patients with MTX-induced renal failure, especially if it is administered within 48-72 h after the start of MTX infusion.

For the future, it might be helpful to differentiate between patients with extremely high MTX concentrations and severe renal failure and patients with moderately high MTX concentration and renal failure. Since additional injections at CPDG₂ did not result in a significant further decrease of MTX levels, one initial high dose of 100 µg kg⁻¹ CPDG₂ for patients with extremely high MTX concentrations (for example, >100 µmol l⁻¹ at 48 h after MTX start and renal failure) may be more effective. For the patients with moderately high MTX levels and renal failure, the application of 50 µg kg⁻¹ seems to be sufficient. Due to the high costs of the drug, it may be discussed whether an application of 25 µg kg⁻¹ CPDG₂ would not be sufficient to lower the MTX levels to nontoxic concentrations in patients with marginal high MTX concentrations and renal failure.

The therapy with CPDG₂ should always be accompanied by a sufficient hydration, alkalisation and a long-term LV therapy, in order to counterbalance the intracellular MTX. Haemofiltration or haemodialysis might be helpful in oliguric or anuric renal failure. In individual cases with renal failure, severe and lethal courses may occur despite a CPDG₂ application, in particular if delayed CPDG₂ administration did not prevent overt toxicity.

ACKNOWLEDGEMENTS

We thank the following hospitals for the transmission of patient data and partly also for serum samples: Department of Pediatric Hematology/Oncology of: University of Brussels, Belgium; University of Prague, Czech Republic; University of Dresden, Düsseldorf, Essen, Freiburg, Hamburg; Hannover, Heidelberg; Kiel; Mainz, Münster, Saarland, Würzburg, all Germany; University of Tromso, Norway; University of Bratislava, Slovakia; Department Medical Oncology of: University of Montpellier, France; University of Bensaon, France; University of Bonn, Cologne, Halle, all Germany; University of Navarra, Spain; Hospital Laennec Paris, France; Carl-Thiem Clinical Center Cottbus, City Children’s Hospital Kassel, Children’s Hospital Kohlhof Neunkirchen, Clinical Center Schwerin, Robert Bosch Hospital Stuttgart, Hospital “Barmherzigen Brüder” Trier, all Germany; Children’s Hospital ‘Aglia Kyriakou’, Athens, Greece; Cantonal Hospital, Basel, Switzerland; Children’s Cantonal Hospital, Geneva, Switzerland. This study was supported by Protherics Plc, Salisbury, UK.

REFERENCES

Abelson HT, Ensminger W, Rosowsky A, Uren J (1978) Comparative effects of citrovorum factor and carboxypeptidase G₁ on cerebrospinal fluid-methotrexate pharmacokinetics. Cancer Treat Rep 62: 1549 – 1552

Adamson PC, Balis FM, McCully C, Godwin KS, Poplack DG (1992) Monitoring high-dose methotrexate in plasma. Clin Chem 38: 39 – 44

Ahmad S, Shen FH, Bleyer WA (1978) Methotrexate induced renal failure and ineffectiveness of peritoneal dialysis. Arch Intern Med 138: 1146 – 1147

Albertoni F, Rask C, Eksborg S, Poulsen JH, Pettersson B, Beck O, Schroeder H, Peterson C. (1996) Evaluation of clinical assays for measuring high-dose methotrexate in plasma. Clin Chem 42: 39 – 44

Allegre CJ (1990) Antifolates. In Cancer Chemotherapy Chabner BA, Collins JM (eds) pp 110 – 153. Philadelphia: Lippincott

Bertino JR, Skell R, Makulu D, McIntosh S, Uhoch J, Chabner B (1974) Initial clinical studies with carboxypeptidase G₁ (CPG₁) a folate depleting enzyme. Clin Res 22: 483A

Brandsteterova E, Seresova O, Miertus S, Reichelova V (1990) HPLC determination of methotrexate and its metabolites in serum. Neoplasma 37: 395 – 403

Chabner BA, Johns DG, Bertine JR (1972) Enzymatic cleavage of methotrexate provides a method for prevention of drug toxicity. Nature 239: 395 – 397

Ensminger WD, Frei E (1977) The prevention of methotrexate toxicity by the thymidine infusions in humans. Cancer Res 37: 1857 – 1863

Frei III E, Blum RH, Pitman SW, Kirkwood JM, Henderson IC, Skarin AT, Mayer RJ, Bast RC, Garnick MB, Parker LM, Canellos GP (1980) High dose methotrexate with leucovorin rescue: rationale and spectrum of antitumor activity. Am J Med 68: 370 – 376

Gibson TP, Reich SD, Krumlovsky FA, Ivanoch P, Gonczy C (1978) Hemoperfusion for methotrexate removal. Clin Pharmacol Ther 23: 351 – 355

Goldman ID (1975) Membrane transport of methotrexate and other folate compounds: relevance to rescue protocols. Cancer Chemother Rep 6: 63 – 72
Goldman P, Levy CC (1968) The enzymatic hydrolysis of folate analogues. *Biochem Pharmacol* 17: 2265 – 2270
grem JL, King SA, Sorensen JM, Christian MC (1991) Clinical use of thymidine as a rescue agent from methotrexate toxicity. *Invest New Drugs* 9: 281 – 290
howell SB, Ensminger WD, Krishan A, Frei E (1978) Thymidine rescue of high-dose methotrexate in humans. *Cancer Res* 38: 325 – 330
howell SB, Herbst K, Boss GR, Frei E (1980) Thymidine requirements for the rescue of patients treated with high-dose methotrexate. *Cancer Res* 40: 1824 – 1839
juergens H, Beron G, Winkler K (1983) Toxicity associated with combination chemotherapy for osteosarcoma: a report of the cooperative osteosarcoma study (COSS 80). *J Cancer Res Clin Oncol* 106(Suppl): 14 – 18
levy CC, Goldman P (1967) The enzymatic hydrolysis of methotrexate and folic acid. *J Biol Chem* 242: 2933 – 2938
minton NP, Atkinson T, Sherwood RF (1983) Molecular cloning of the *Pseudomonas* carboxypeptidase G2 gene and its expression in *Escherichia coli* and *Pseudomonas putida*. *J Bacteriol* 156: 1222 – 1227
National Cancer Institute Common Toxicity Criteria (NCI CTC version 2.0). http://ctep.cancer.gov/forms/CTCv20_4-30-992.pdf
Pinedo HM, Zarharko DS, Bull JM, Chabner BA (1976) The reversal of methotrexate cytotoxicity to mouse bone marrow cells by the leucovorin and nucleosides. *Cancer Res* 36: 4416 – 4424
Relling MV, Stapleton FB, Ochs J, Jones DP, Meyer W, Wainer IW, Crom WR, McKay CP, Evans WE (1988) Removal of methotrexate, leucovorin, and their metabolites by combined hemodialysis and hemoperfusion. *Cancer* 62: 884 – 888
saland JM, Leavey PJ, Rash RO, Hansch E, Arbus GS, Quigley R (2002) Effective removal of methotrexate by high-flux hemodialysis. *Pediatr Nephrol* 17: 825 – 829
schrappe M, for the ALL-BFM study committee (1995) *ALL-BFM-95-Protokoll* Hannover, Germany
Sherwood RF, Melton RG, Alwan SM, Hughes P (1985) Purification and properties of carboxypeptidase G2 from *Pseudomonas* sp. strain RS-16. Use of a novel triazine dye affinity method. *Eur J Biochem* 148: 447 – 453
van den Bongard H, Mathot R, Booger W, Schornagel JH, Soesan M, Schellens JH, Beijnen JH (2001) Successful rescue with leucovorin and thymidine in a patient with high-dose methotrexate induced acute renal failure. *Cancer Chemother Pharmacol* 47: 537 – 540
widemann BC, Balis FM, Murphy RF, Sorensen JM, Montello MJ, O’Brien M, Adamson PC (1997) Carboxypeptidase-G2, thymidine, and leucovorin rescue in cancer patients with methotrexate-induced renal dysfunction. *J Clin Oncol* 15: 2125 – 2134
widemann BC, Hetherington ML, Murphy RF, Balis FM, Adamson PC (1995) Carboxypeptidase-G2 rescue in a patient with high dose methotrexate-induced nephrotoxicity. *Cancer* 76: 521 – 526
widemann BC, Sung E, Anderson L, Salzer WL, Balis FM, Monitjo KS, McCully C, Hawkins M, Adamson PC (2000) Pharmacokinetics and metabolism of the methotrexate metabolite 2,4-diamino-N(10)-methylpterioic acid. *J Pharmacol Exp Ther* 294: 894 – 901
zoubek A, Zaunschirm HA, Lion T, Fischmeister G, Vollhofer G, Gadner H, Pillwein K, Schalhorn A, Bode U (1995) Successful carboxypeptidase G2 rescue in delayed methotrexate elimination due to renal failure. *Pediatr Hematol Oncol* 12: 471 – 477

© 2005 Cancer Research UK