Extremely delayed elimination of methotrexate in a young man with osteosarcoma: A case study demonstrating an association with impaired renal function.

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Abstract— Background: Methotrexate (MTX) is one of the most widely used anti-cancer agents, and administration of high-dose methotrexate followed by leucovorin (LV) rescue therapy is an important component in the treatment of a variety of cancers. High-dose MTX is thought to be safe for administration in patients with normal renal function with concomitant alkalinization, hydration, and pharmacokinetically-guided leucovorin rescue. However, acute renal failure and other adverse outcomes are unavoidable under certain circumstances. High-dose methotrexate (HDMTX)-induced renal dysfunction can be a life threatening event, because it delays methotrexate excretion, thereby exacerbating the other toxicities of MTX. High-dose methotrexate-induced renal dysfunction continues to occur in patients with osteosarcoma who are treated on clinical protocols despite optimal supportive care. Approximately, 1.8% of patients with osteosarcoma, who received HDMTX may develop nephrotoxicity of various grade carrying considerable mortality rate. High-dose methotrexate-induced renal dysfunction can be life threatening, because it delays methotrexate excretion, thereby exacerbating the other toxicities of methotrexate. HDMTX-induced nephrotoxicity has been usually managed with high-dose leucovorin, dialysis-based methods of MTX removal, thymidine, and recently by administration of the recombinant enzyme, carboxypeptidase-G2 (CPDG2), which cleaves MTX to inactive metabolites. Objective: The aim of this paper is to describe the case of an adult Caucasian male patient with osteosarcoma who presented with extremely delayed MTX clearance after high-dose administration conducted according to the EURAMOS protocol. In the present case, We discuss also the fate of the patient where delayed MTX excretion was a big challenge and finally managed using supportive measures including high doses of leucovorin and very effectively CPDG2 without having to adopt other invasive procedures like dialysis.

Keywords—Osteosarcoma, High-dose MXT, Nephro-Toxicity

I. INTRODUCTION

Osteosarcoma (osteogenic sarcoma) is the most common type of primary bone cancer most occurring in children and young adults with peak incidences in adolescence and at age >60 years, but can occur at any age.[1] Methotrexate (MTX), a
classic antifolate is one of the most widely used and well studied anticancer agents, where the administration of MTX doses ≥ 1000 mg/m² combined with leucovorin (LV) rescue is defined as high-dose methotrexate (HDMTX) and is an important component of treatment for a variety of malignancies, including osteosarcoma. Todate, a very high-dose methotrexate usually >1 g/m² administered as an intravenous infusion remains an important component in the treatment of variety of cancers in particular for osteosarcoma, but this HDMTX treatment schedule carries a risk of nephrotoxicity among others. Data from a number of studies performed in the 1970s showed that a sustained elevation of serum MTX concentrations at 24 h (≥5 μmol/L), 48 h (≥1 μmo/L) and 72 h (≥0.1 μmo/L) after the start of the MTX infusion is considered to be toxic referring to the usual serum MTX level <0.1 μmol/L 48 h after HDMTX administration. In the era of optimal supportive care the incidence of grade 3–4 ARF after HDMTX has reportedly decreased markedly in solid-cancer patients. Although HDMTX-associated severe acute renal failure (ARF) is an infrequent, the very high doses of MTX generally used in solid-cancer patients such as with osteosarcoma, who receive a MTX dose >8 g/m², the risk of severe ARF may be certainly higher. According to large case series, the reason why an individual patient comes prone to develop ARF after HDMTX despite modern supported care remains unexplained in the majority of cases (1-3). But drug drug interactions are mostly unrecognized or overlooked. In case of our patient the role of pre treatment with Cis-platin in the near past before the initial therapy with MXT may be the partial explation for the precipitation of acute renal dysfunction grade 2-3 (Renal toxicity was graded using World Health Organization criteria (Grade 1, serum creatinine levels < 1.5 × ULN; Grade 2, 1.5–3.0 × ULN; Grade 3, 3.1–6.0 × ULN; and Grade 4, > 6.0 × ULN) Although the incidence and mortality of HDMTX-induced renal dysfunction appear to have decreased significantly since the 1970s, nephrotoxicity continues to occur and may be fatal. Therefore, in situations, where usual care fails in patients with delayed MTX excretion and high plasma MTX concentrations, other measures like treatment by CPDG₂ should be considered to lower plasma MTX concentrations rapidly and efficiently as previously recommended. The median time to recovery of renal function for a number of patients, as defined by the individual studies, was 16 days (range, 4–48 days), where treatment of patients did not include carboxypeptidase-G₂. According to published literature reviewed from 1977 to 2002 on recovery of renal function in patients with methotrexate-induced renal dysfunction, the median time to recovery of renal function for a number of patients, as defined by the individual studies, was 16 days (range, 4–48 days), where treatment of patients did not include carboxypeptidase-G₂. [3, 8]

II. CASE DESCRIPTION

A 37-year-old Caucasian male had been treated initially had been treated initially with a combination of doxorubicin and cisplatin for proven diagnosis of osteosarcoma. Just after a month later, the patient was scheduled for methotrexate treatment according to the EURAMOS (European and American Osteosarcoma Study Group) protocol, a joint protocol of four of the world’s leading multi-institutional osteosarcoma groups; which uses a dose of 12 g/m² over a 4-hour infusion and repeated with 11.34 g/m². The concentration of MXT determined by fluorescent polarization immunoassay (FPIA) method 6 hours post-infusion showed levels within expected range. However,
measurements taken 24 hours post-dose and later were extremely high. This indicated poor elimination and was also confirmed by significantly elevated serum creatinine (Fig. 1) as well as blood urea nitrogen. Drug plasma level monitoring was continued on a daily basis as per protocol guidelines until the level reached less than 0.1uM. This took one month +8 days from initial MXT administration.

**Fig. 1.** Extremely delayed methotrexate (MXT) elimination corresponding with serum creatinine levels (S-Cr) and persistence of high blood urea nitrogen (BUN) demonstrating significant renal function impairment.

Taking into consideration that with high-dose methotrexate, toxic concentrations are generally considered to be: ≥ 5 µmol/L at 24 hours after the dose, ≥ 0.5 µmol/L at 48 hours, and ≥ 0.055 µmol/L at 72 hours; we declare our findings as potentially extremely toxic levels. The test results are used to guide the amount and timing of leucovorin (folinic acid) given as a "rescue" treatment, but the effect of the rescue therapy was not satisfactory in this case. Finally, carboxypeptidase-G2 (CPDG2) has been used with significant effect in reducing the drug level by 80 % of the previously recorded value. However, renal function and further drug elimination were lagging for several days. BUN and serum creatinine were not restored to normal until after a month. It took more than one month to get the drug plasma level of 0.11 umol/l as illustrated above (Fig.1). At the time of very high drug levels, the patient also manifested with significantly high activity of liver aminotransferases, namely ALT (up to 30 U/L). AST was moderately increased (4 U/L) and both were shortly thereafter restored. This was in contrast to BUN and serum creatinine levels which remained abnormal over the course of a month. Thrombolytic and leukocyte profiles were also demonstrably unstable throughout follow-up until the elimination of the drug (Fig. 2). Prominent leukopenia was observed in the week after drug exposure; whereas thrombocytopenia was a few days earlier (Fig. 2). Both events of leukopenia and thrombocytopenia had several phases demonstrating instability of the blood count in association with a prolonged elevated level of methotrexate exposure.

**Fig. 2.** Thrombolytic and leukocyte profiles demonstrating instability of the blood count associated with a prolonged elevated level of methotrexate exposure.

### III. DISCUSSION

Some acute toxicities like liver toxicity manifesting with ALT and AST transient elevation were reported in the past as reversible without further concern as self limited [9] as also has been observed in our patient case. Despite advanced management and care measures, high-dose MTX-induced renal dysfunction continues to occur in approximately 2% of patients with osteosarcoma treated in clinical trials. [3] Early recognition and treatment of MTX-induced renal dysfunction are essential in preventing potentially life-threatening toxicities; especially myelosuppression, renal failure, mucositis and dermatitis. In addition to conventional treatment approaches, dialysis-based methods have been used to remove MTX with limited effectiveness. More recently, CPDG2, a recombinant bacterial enzyme that rapidly hydrolyzes MTX to inactive metabolites, has become available for the treatment of MTX-induced renal dysfunction. [4] Certain circumstances like ascites and packed red blood cell infusion may
function as a reservoir and enhance prolonged high level exposure to methotrexate during a high-dose regimen, but our patient had only suffered mild pleural effusion, not ascites, to serve as a possible reservoir. Some previously published studies also identified several clinical variables that influence MTX disposition that, when modified, can reduce the frequency of high-risk MTX concentrations and toxicity [10]. Clearance is exceptionally variable in individuals and association with age and gender has been also documented [11]. However, none of these variables explain the extremely delayed elimination of the drug in our patient. Similar to other antimetabolites, critical determinants of MTX cytotoxicity is not only drug concentration, but also the duration of exposure. High concentrations of MTX may be well-tolerated for brief periods of time; whereas prolonged exposure to low concentrations can result in life-threatening toxicity. The type of toxicity observed with MTX is also a function of this concentration–time dependence. Exposure to millimolar concentrations of MTX for minutes to hours may lead to acute renal, central nervous system, and liver toxicity. Exposure to MTX concentrations as low as 0.01 and 0.005 μM for > 24 hours may result in bone marrow and gastrointestinal epithelial toxicity, respectively [12]. The MTX-induced renal dysfunction is believed to be mediated by the precipitation of MTX and its metabolites in the renal tubules [13-15] or via a direct toxic effect of MTX on the renal tubules [16]. Urinary NAG:creatinine ratio in our patient after 3 weeks continued to demonstrate abnormality correlating to delayed function reversibility since more than 90% of MTX is cleared by the kidneys [17]. MTX is poorly soluble at acidic pH and its metabolites, 7-OH-MTX and DAMPA, are six- to tenfold less soluble than MTX [13, 18]. An increase in urine pH from 6.0 to 7.0 results in a five- to tenfold greater solubility of MTX and its metabolites; a finding that underlies the recommendation of i.v. hydration (2.5–3.5 litres of fluid per m² per 24 hours, beginning 12 hours before MTX infusion and continuing for 24–48 hours) and urine alkalinization (40–50 mEq sodium bicarbonate per liter of i.v. fluid prior to, during, and after the administration of high-dose MTX as performed in the present case. Several drugs have also been associated with increased toxicity when co-administered with MTX [6]. The most significant interactions involve agents that interfere with MTX excretion, primarily by competing for renal tubular secretion, such as: probenecid, salicylates, sulfisoxazole, penicillins, and nonsteroidal anti-inflammatory agents [19], but all were excluded in the present case. MTX-induced renal dysfunction results in sustained, elevated plasma MTX concentrations; which in turn may lead to ineffective rescue by leucovorin and a marked enhancement of MTX’s other toxicities; especially myelosuppression, mucositis, hepatitis, and dermatitis [20, 21]. Previous studies demonstrated that: (a) sustained elevation of plasma MTX concentrations at 24 hours (> 5–10 μM), 48 hours (> 1.0 μM), and 72 hours (> 0.1 μM) after administration of MTX are predictive for the development of toxicity; (b) in the absence of elevated plasma MTX concentrations, the risk for the development of MTX-associated toxicities is minimal; (c) in most circumstances, the development of MTX-associated toxicities can be ameliorated or prevented when patients with elevated plasma MTX concentrations receive pharmacokinetically-guided doses of LV rescue. These studies resulted in uniform institution of aggressive hydration, alkalinization, and pharmacokinetically-guided LV. Nomograms guiding the duration and degree of rescue with LV based upon plasma MTX concentrations as a function of time of drug administration were developed and are being used in ongoing clinical trials that administer high-dose methotrexate [22]. Goren et al., [23] found that analysis of the changes in these sensitive markers of renal tubular damage permitted detection of subclinical methotrexate-induced nephrotoxicity. According to the authors, persistent rises in NAG as well as increased serum creatinine levels in patients with osteogenic sarcoma who were receiving combination chemotherapy that included 12 doses of methotrexate (12 g/m²) was associated with doses of methotrexate that followed the administration of cisplatin (400 mg/m²), while the biphasic pattern of NAG excretion observed in patients suggests more than one mechanism of methotrexate-induced nephrotoxicity. Thus, monitoring renal tubular damage in patients who are receiving methotrexate in combined drug regimens would provide useful information. In the study to determine the risk of impaired excretion of methotrexate in patients with osteosarcoma, who were also receiving cisplatin, it has been found that MTX clearance was impaired in patients with urinary NAG concentrations greater than 1.5 U/mmol creatinine or greater than 50% increase in serum creatinine relative to the pre-therapy level, were approximately 30 times more likely to have MTX half-lives greater than 3.5 hours than were patients with lower values for these markers (i.e., MTX clearance was always impaired if both markers were elevated) [24]. These findings demonstrate that urinary NAG and serum creatinine levels, measured before MTX administration, can be used to identify patients who will have difficulty clearing the drug and thus can be used to guide
rescue measures including: highly effective carboxyptidase-G2, thymidine, and leucovorin in patients at high risk for developing life-threatening methotrexate toxicity after the onset of methotrexate-induced nephrotoxicity and delayed methotrexate excretion [25]. In the case described here, the NAG:creatinine ratio was abnormal several days post-MXT exposure. Moderate hypokalemia and hyponatremia observed later on may also be explained by poor tubular function. Over all Early recognition of the failure to eliminate excess MTX may help start of more effective rescue treatment such as CPDG2 application recently published as better alternative.[5] Based on our literature review and information available from other databases [3] , approximately 1.8% of patients with osteosarcoma who are treated with HDMTX develop significant nephrotoxicity at some time during treatment, and the mortality among these patients is estimated at 4.4%. Limitations of this estimate include the following: nephrotoxicity was not defined uniformly across studies, trials were not designed to capture data concerning nephrotoxicity comprehensively, and few published studies included a comprehensive description of MTX-related toxicities. In addition, only patients entered on clinical trials, who may be more likely to receive optimal supportive care, were included in the estimate. Therefore, the incidence of MTX-induced renal dysfunction among all patients receiving HDMTX may be higher. Myelosuppression occurs in 28 per cent of the patients and in 8 per cent of the courses and usually results from delayed MTX excretion secondary to mild reversible nephrotoxicity.[26] However, the manifestation in a present case osteosarcoma patient was not relevant for significant haematotoxicity. Aggressive hydration, urine alkalinization aiming to keep urine pH 7 to 8.5, Leucovorin administration, close monitoring of urine output, fluid balance avoiding negative balance, serial MTX levels and Serum Cr monitoring are among preventive measures against HDMTX potential nephrotoxicity. [27] In some individuals preventive measures sometimes fail to protect patients from MTX-induced nephrotoxicity. In such cases, standard approaches namely, hydration, urine alkalinization, and leucovorin rescue are also key to managing patients who develop HDMTX-induced renal dysfunction. Additional measures include extracorporeal interventions such as hemofiltration and dialysis, as well as pharmacotherapy with glucarpidase. Glucarpidase (carboxyptidase G2) , which was approved by the US Food and Drug Administration (FDA) in January 2012 for the treatment of cases with plasma MTX concentrations (> 1 μM) in patients with delayed MTX clearance due to impaired kidney function despite standard leucovorin application. [28]

IV. CONCLUSION

Based on the case demonstrated, We recommend prompt recognition of patients with poor elimination of after administration of high-dose methotrexate and to start effective rescue therapy including glucarpidase (carboxyptidase G2) for its effective elimination of MXT to avoid further deterioration of health and to improve overall outcomes.

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REFERENCES