Significantly delayed elimination of methotrexate in osteosarcoma patient: an association with impaired renal function deterioration.

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Abstract: - Methotrexate is one of widely used anti-cancer agent. High-dose methotrexate (HDMTX) followed by leucovorin rescue therapy is an important component in the treatment of a variety of cancers including osteosarcoma. Unfortunately, acute renal failure and other adverse effects are hardly avoidable. Despite advanced care measures, HDMTX-induced renal dysfunction continues to occur in approximately 2% of patients with osteosarcoma. The aim of this contribution is to describe the case of an adult Caucasian male patient with osteosercoma, who demonstrated extremely delayed MTX elimination after HDMTX treatment according to the European and American Osteosarcoma Study Group (EURAMOS) joint protocol using an initial dose of 12 g/m^2 over a 4-hour infusion. We also discuss the fate of the patient where delayed MTX excretion was a great challenge and how promptly recognition of patients with poor MXT elimination is of vital importance to start effective rescue therapy for better overall outcomes.

Key-Words: - Osteosarcoma, methotrexate-induced nephrotoxicity, rescue therapy, leucovorin, carboxypeptidase-G_2

1 Introduction

As Osteosarcoma (osteogenic sarcoma) is the most common type of primary bone cancer in children and young adults. Bimodal peak incidences occur in adolescence and at ages > 60 years, but can occur at any age.[1] Methotrexate (MTX), a classic anti-folate, is one of the most widely used and well-studied chemotherapeutic agents and is an important component of treatment for a variety of malignancies, including osteosarcoma.[2] Treatment often involves high-dose methotrexate (HDMTX); which is defined as intravenous administration of MTX doses $\geq$ 1000 mg/m^2 combined with leucovorin (LV) rescue emains an important component in the treatment of osteosarcoma, while carrying amongst other risks, the possibility of nephrotoxicity..[3] Under optimal supportive care, the incidence of grade 3–4 acute renal failure (ARF) after HDMTX administration has markedly decreased in solid tumor cancer patients.[4] Although HDMTX-associated severe ARF is an infrequent morbidity, those receiving >8 g/m^2, such as osteosarcoma patients, are at increased risk. According to large case series, the reason why an individual patient becomes prone to develop ARF after HDMTX, despite modern supportive care, remains unexplained in the majority of cases.[1-5] Although the incidence and mortality of HDMTX-induced renal dysfunction appear to have significantly decreased since the 1970s [6], nephrotoxicity continues to occur and may be fatal. According to reviewed literature published from 1977-2002, the median time of renal function recovery from MTX-induced renal dysfunction for those who were not treated with carboxypeptidase-G_2 was 16 days (Range: 4–48 days).[7] Therefore, in situations when usual care fails in patients with delayed MTX excretion and plasma MTX concentrations continue to be elevated, other measures such as $CPDG_2$ treatment should be considered in order to lower plasma MTX concentrations rapidly and efficiently as previously

recommended.[3] The aim of this paper is to describe the case of an adult Caucasian male patient with osteosercoma who presented with extremely delayed MTX clearance after high-dose administration conducted according to the EURAMOS protocol.

2 Case description

A 37-year-old Caucasian male had been initially treated with a combination of doxorubicin and cisplatin for proven diagnosis of osteosarcoma. Just a month later, the patient was scheduled for high-dose methotrexate treatment according to the European and American Osteosarcoma Study Group (EURAMOS) joint protocol EURAMOS protocol; which uses a dose of 12 g/m^2 over a 4-hour infusion and repeated with 11.34 g/m^2. The MXT plasma concentrations determined by fluorescent polarization immunoassay (FPIA) method 24 hours post-infusion and later were extremely high, indicating poor elimination in association with significantly elevated serum creatinine as well as blood urea nitrogen(BUN) level.(Fig 1.) High drug levels were also accompanied by abnormal aminotransferases, namely ALT (up to 30 U/L). AST moderately increased (4 U/L), but shortly restored. 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 as illustrated in Fig.1.

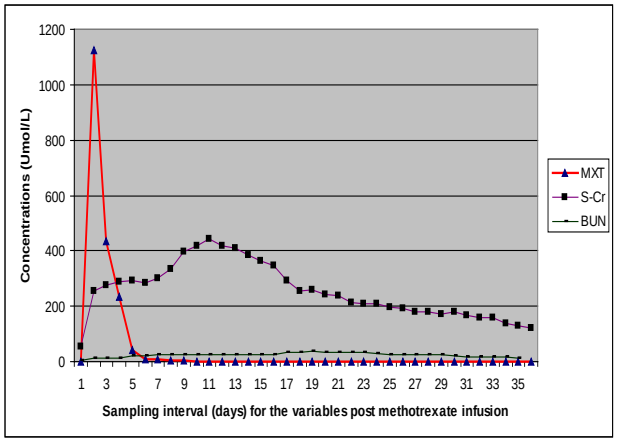

Fig. 1. Extremely delayed methotrexate (MXT) elimination corresponding with serum creatinine levels (S-Cr) and persistently high blood urea nitrogen (BUN) demonstrating evident renal function impairment.

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-G_2 ($CPDG_2$) has been used with significant effect in reducing the drug level by 80 % of the previously recorded value. Liver aminotransferases ,which were elevated at the time of very high drug levels were also shortly thereafter restored. This was in contrast to BUN and serum creatinine levels, which remained abnormal over the course of a month. As renal function and further drug elimination were lagging, it took more than one month to achieve the low drug plasma level of 0.11umol/l as illustrated (Fig.1.). 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 complete elimination of the drug (Fig. 2). Significant 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 exposure to high level of methotrexate.

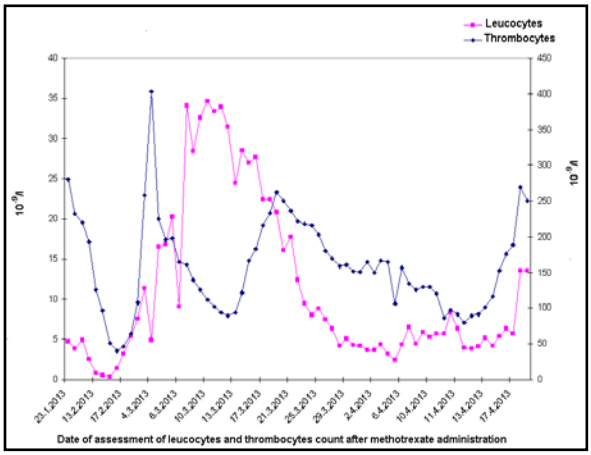

Fig. 2. Thrombolytic and leukocyte profiles demonstrating instability of the blood count associated with a prolonged exposure to high concentration of methotrexate

3. Discussion

Although it has recognized efficacy, high dose MXT MTX is associated with several adverse effects including nephrotoxicity, hepatotoxicity, neurotoxicity mucositis, and pulmonary toxicity

among others.[8, 9] 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. Early recognition and treatment of MTX-induced renal dysfunction are essential in preventing potentially life-threatening toxicities; especially myelosuppression and renal failure. 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. Co-administration of some drugs have been also reported to delay elimination of plasma methotrexate [10-14], but this was not the case in our patient. 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.[15] Based on the results of the study to investigate the relationships between pretreatment folate concentrations, MTX pharmacokinetics and acute toxicities following high-dose MXT therapy it has been suggested that 7-OH-MTX may be involved in the development of high-dose MTX hepatic toxicity.[16] Unfortunately 7-OH-MTX, a metabolite of the parent drug , which is also associated with nephrotoxicty .[17] has not been measured in our case. It is also known that, there might be interference of other minor metabolite namely 2,4-diamino-N10-methylpteroic acid (DMPA) to falsely increase the MXT plasma level during the assay, but this may not be significant at the presence of MXT itself. [18] Clearance is exceptionally variable in individuals and association with age and gender has been also documented.[19] However, none of these variables explain the extremely delayed elimination of the drug in our patient. Nevertheless the long time detection of MXT after one cycle dose of MXT is not well explained, although the difficulty of measuring accurately MXT plasma levels after doses of carboxypeptidase-G2 exists.[20] 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.[21]. The MTX-induced renal dysfunction is believed to be mediated by the precipitation of MTX and its metabolites in the renal tubules (.[7, 22, 23] or via a direct toxic effect of MTX on the renal tubules.[24] 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.[25] Methotrexate is poorly soluble at acidic pH and its metabolites, 7-OH-MTX and DAMPA, are six- to tenfold less soluble than MTX.[22, 26] An increase in urine pH from 6.0 to 7.0 results in a five- to eightfold 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 m^2 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. 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 .[27], 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.[28, 29] Nomograms guiding the duration and degree of rescue therapy with leucovorin based upon plasma MTX concentrations as a function of time of drug administration were developed and are being used in clinical trials that administer high-dose methotrexate.[30] Changes in sensitive markers of renal tubular damage like rise in urinary N-acetyl-β-D-glucosaminidase (NAG) level may

allow detection of subclinical methotrexate-induced nephrotoxicity. In patients with osteogenic sarcoma who were receiving combination chemotherapy that included 12 doses of methotrexate (12 g/m^2) persistent rise in NAG level was associated with doses of methotrexate that followed the administration of cisplatin (400 mg/m^2), while the biphasic pattern of NAG excretion observed in patients suggests more than one mechanism of methotrexate-induced nephrotoxicity.[31] 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, 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.[32].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 in patients at high risk for developing life-threatening methotrexate toxicity after the onset of methotrexate-induced nephrotoxicity and delayed methotrexate excretion.[3] 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. Overall, therapy monitorning and early recognition and treatment of MTX-induced renal dysfunction are essential in preventing potentially life-threatening toxicities; especially myelosuppression and renal failure as a recent report of fatal cases of even low dose methotrexate (MTX) toxicity in patients for other therapeutic indications also warns.[34] In addition to conventional treatment approaches, dialysis-based methods have been used to remove MTX with limited effectiveness. More recently, $CPDG_2$, a recombinant bacterial enzyme that rapidly hydrolyzes MTX to inactive metabolites DAMPA (4-[[2,4-diamino-6-(pteridinyl)methyl]-methylamino]-benzoic acid) and glutamate in patients with delayed MTX excretion has become available for the treatment of MTX-induced renal dysfunction.[5, 35] Although the clear underlying mechanism is to be further elucidated Carboxypeptidase G_2 a zinc-metalloenzyme is employed in cancer chemotherapy for its property of selectively activating nontoxic prodrugs into cytotoxic drugs in tumor, whereas it is also used in the treatment of toxicity following high-dose methotrexate treatment. At molecular level, carboxypeptidase G_2 is known to catalyze the hydrolytic cleavage of C-terminal of glutamate moiety from folic acid and analogues, while recent investigation indicates that additionally, a glutamate residue can interact with a crystallization water molecule in the active site, supporting its activation as a nucleophilic group.[36] Otherwise, other treatment options /strategy like recently published high-dose MXT- free regimen [37] should be considered in osteosarcoma patients if proved by further research and clinical application.

4. Conclusions

Although acute liver toxicity manifesting with transient aminotransferases elevation is reversible, methotrexate-induced nephrotoxicity may be very challenging especially in patients with evidently delayed MTX clearance due to impaired kidney function. Prompt recognition of patients with poor elimination after administration of HDMTX is of vital importance to start effective rescue therapy including $CPDG_2$ to avoid further deterioration of health and to improve overall outcomes.

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Conflict of Interest Disclousure: The corresponding author declares no conflict of interest pertaining to this case report.

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