Kidney Medicine

Methotrexate Crystals on Electron Microscopy of Kidney Biopsy for Acute Kidney Injury

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Acute kidney injury secondary to methotrexate therapy for hematologic malignancies is relatively uncommon. Methotrexate crystals in these cases are rarely seen on kidney biopsy, and in particular, their appearance in tissue prepared for transmission electron microscopy has not been described. A male patient with recurrent primary central nervous system lymphoma received high-dose methotrexate and rituximab for treatment. On day 2 of cycle 3, one day after the infusion of high-dose methotrexate, the patient was found to have high levels of serum methotrexate. Shortly after, he developed acute kidney injury. A kidney biopsy was performed, which showed methotrexate crystals only on tissue submitted for electron microscopy. To our knowledge, this is the first report to characterize methotrexate crystals on toluidine blue-stained thick sections and their ultrastructure on transmission electron microscopy.

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INTRODUCTION

Acute kidney injury (AKI) occurs in ∼2% of patients receiving high-dose methotrexate (hd-MTX) for the treatment of a hematologic malignancy.[1] Injury from MTX is caused by precipitation of crystals in the tubules and also by direct toxic injury to the epithelium. A few reports have characterized the appearance of methotrexate crystals in the urine,[2–5] and on histology at the light microscopic level of kidney biopsies.[3,6–8] However, the appearance of methotrexate crystals on toluidine blue-stained sections and the ultrastructure of methotrexate crystals have not been described.

To our knowledge, this report is the first to describe methotrexate crystals on toluidine blue-stained thick sections and on transmission electron microscopy (TEM) of a kidney biopsy. Unique aspects of MTX crystals are identified that may allow for distinction of MTX from other crystals in kidney biopsies. The case highlights the importance of thorough examination of biopsy tissue for localized features that may be missed on light microscopy alone, leading to an incomplete or erroneous diagnosis, and the lack of sensitivity of urine crystals in suspected cases of MTX-induced AKI.

CASE REPORT

Clinical History and Initial Laboratory Data

A 60-year-old man with biopsy-confirmed recurrent primary central nervous system lymphoma presented for chemotherapy administration of hd-MTX ($8\ g/m^2$/dose × $1.99\ m^2$) and rituximab. He was also found to have a urinary tract infection with extended spectrum β-lactamase *Escherichia coli* and was treated with cefepime, vancomycin, meropenem, and ertapenem at various times throughout his hospitalization.

The patient's baseline serum creatinine levels ranged from 0.7-0.9 mg/dL, with a serum creatinine level of 0.5 mg/dL on day 1 of cycle 3 of the hd-MTX infusion. Twenty-four hours later, his serum MTX level was 20 μmol/L (reference range: 0.01-10 μmol/L); serum creatinine level concurrently increased to 1.4 mg/dL (reference range: 0.5-1.6 mg/dL) and continued to increase, reaching a peak of 2.9 mg/dL on day 7 after MTX (Fig 1). According to the MTXPK.org tool, an MTX serum concentration of 20 μmol/L 24 hours following MTX administration exceeded the population upper limit of 95% (10.93 μmol/L). Despite the high levels, no crystals were seen on microscopic examination of the urine sediment. Because of the temporal relationship between high MTX serum levels and subsequent AKI, the patient was administered glucarpidase on day 5. In the setting of the recent urinary tract infection and treatment with multiple antibiotics, such as cefepime and ertapenem, and the high-dose MTX administration, acute pyelonephritis, acute interstitial nephritis, and methotrexate-associated AKI were in the clinical differential. Considered much less likely causes of the AKI were tumor lysis syndrome, infiltration of the kidney by lymphomatous cells, and thrombotic microangiopathy (Table 1). A kidney biopsy was performed on day 7, 6 days after the patient was infused with high-dose MTX.

Kidney Biopsy

Standard processing of the kidney tissue for light, immunofluorescence, and electron microscopy (EM) was performed. Specifically, for TEM, tissue was fixed in 3% glutaraldehyde, postfixed in 1% osmium tetroxide for 1 hour, and dehydrated. Tissue blocks were embedded in epoxy resin. Semithin sections (1-2 micron) of each block were cut and stained with toluidine blue, a standard stain used in the EM technique, which allows for visualization of morphology and selection of appropriate areas for further evaluation on thin sections (70-100 nm) by TEM. The thin sections were stained with 12.5% uranyl acetate and lead

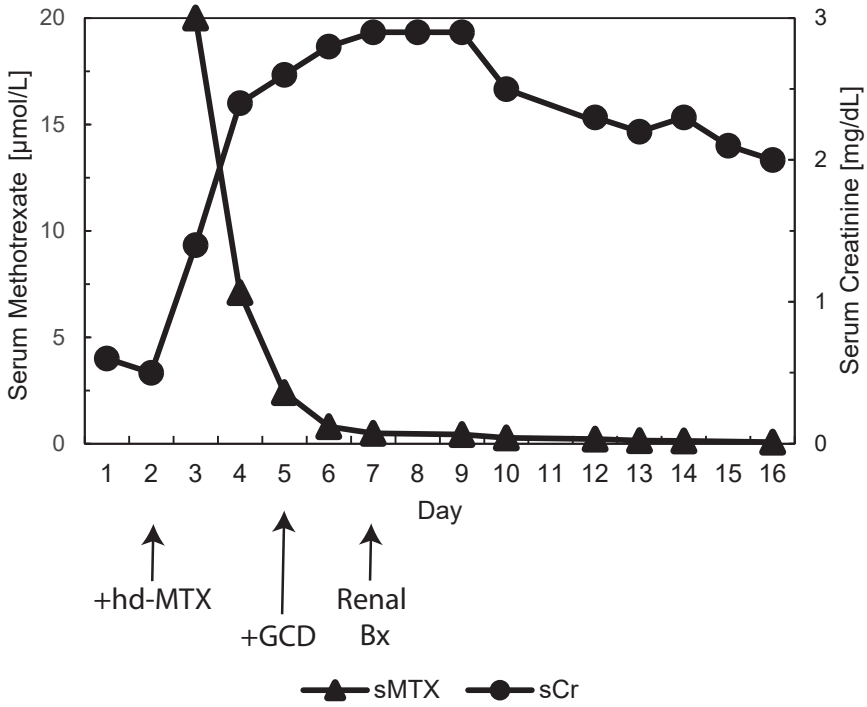

Figure 1. Kidney function progression in relation to methotrexate serum levels. High-dose methotrexate was administered on day 2, glucarpidase (GCD) was administered on day 5, and a kidney biopsy was performed on day 7. *Note:* Conversion factor for serum creatinine levels in mg/dL to µmol/L, ×88.4.

citrate. The sections were examined with a JOEL TEM (JEM-1400) and images acquired with an AMT Nano-Sprint12 camera.

Formalin-fixed, paraffin-embedded sections stained with hematoxylin & eosin (H&E), periodic acid–Schiff, periodic acid–Schiff methenamine silver, and trichrome contained renal cortex and medulla with up to 11 glomeruli, one of which was obsolescent. Patent glomeruli were unremarkable. Tubules showed focal acute injury manifest as cytoplasmic vacuolation, thinning, simplification, and cell sloughing. There was scant interstitial inflammation, comprised of lymphocytes and rare eosinophils, associated with mild edema. Arteries showed moderate intimal fibrotic thickening; arterioles were unremarkable. No crystals or polarizable material were identified on light microscopy.

Immunofluorescence microscopy showed no immune deposits. The frozen sections were examined under polarized light and lacked birefringent material.

Toluidine blue-stained thick sections, which are routinely used in preparation for EM, demonstrated numerous intratubular annular-shaped crystals that exhibited various shades of purple coloration; occasional casts showed a dark purple rim at the periphery of the material. The material demonstrated birefringence on polarization (Figs 2A-D); this property is also a characteristic of MTX crystals identified on formalin-fixed, paraffin-embedded sections examined by light microscopy, illustrating that standard processing of kidney tissue for EM does not affect this characteristic finding. Ultrastructurally, tubule lumens corresponding to the areas seen on toluidine blue-stained sections contained amorphous material on low magnification, usually in a lobulated configuration with low electron density. Occasional cast material had a rim of slightly greater density. On high magnification, an unusual substructure was noted, consisting of randomly arranged, short, straight to wavy fibers or lucent overlapping plaques, correlating with the crystal material noted on the thick sections (Figs 2E-H).

Table 1. Differential Diagnosis and Treatment of AKI in the Setting of High-Dose Methotrexate Administration for Lymphoma

Condition	Treatment
Methotrexate-associated acute tubular injury	Glucarpidase, IV hydration, urine alkalinization
Acute interstitial nephritis	Cessation of offending agent(s) and steroids
Acute pyelonephritis	Antibiotics
Lymphomatous infiltration of the kidney	Treatment of lymphoma and steroids
Tumor lysis syndrome	Rasburicase, IV hydration, and possible dialysis
Thrombotic microangiopathy	Cessation of offending agent, work-up for complement-associated abnormality, and possible eculizumab

Diagnosis

Although crystals were not seen on urine sediment nor were they identified on light microscopy, material

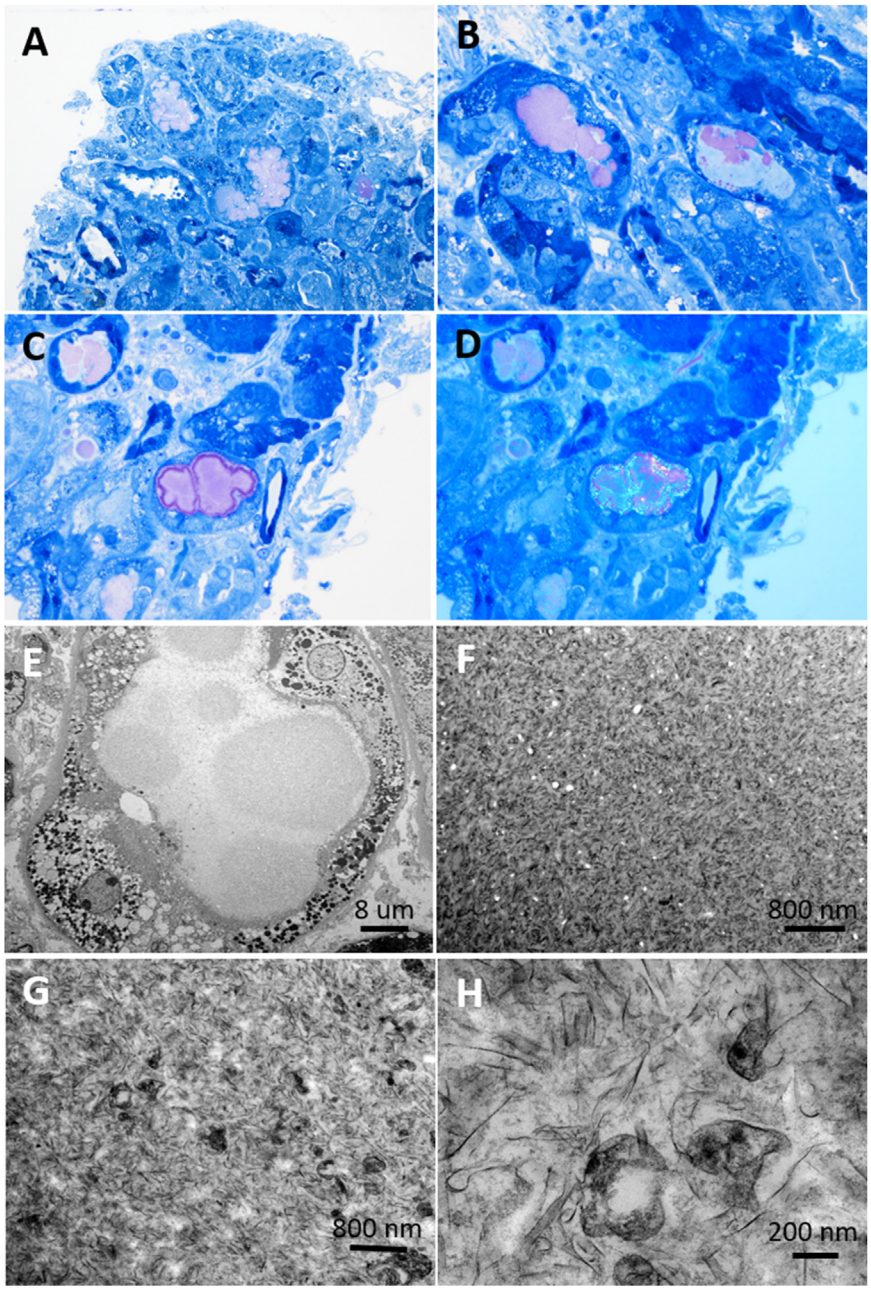

Figure 2. Toluidine blue-stained thick sections and transmission electron micrographs (TEM) of methotrexate crystals. Light violet intratubular crystals were present on thick sections. Original magnification, ×200 (A, toluidine blue). Deeper violet to purple intratubular crystals were present on thick sections. Original magnification, ×200 (B, toluidine blue). Crystals can show a dark purple rim with a central pale area. Original magnification, ×400 (C, toluidine blue). Crystals show birefringence using polarized light. Original magnification, ×400 (D, toluidine blue, polarized). Tubule containing amorphous lobulated, pale electron-dense material. Original magnification, ×2,000 (E, TEM). Luminal material is comprised of randomly arranged, short, straight to wavy fibers. Original magnification, ×25,000 (F and G, TEM). In some foci, the material has an appearance of overlapping plaques. Original magnification, ×80,000 (H, TEM).

consistent with MTX crystals was identified on the toluidine blue-stained thick sections and on the EM—a finding that has not been described before. The tubulointerstitium contained scant inflammation, making acute pyelonephritis or acute interstitial nephritis less likely to explain the patient's increased creatinine levels. No evidence of thrombotic microangiopathy or infiltration by lymphoma was identified. Therefore, the biopsy diagnosis was MTX associated AKI.

Clinical Follow-up

In addition to treating the patient with glucarpidase, the primary team had also treated the patient with oral prednisone for presumed acute interstitial nephritis. One

month after hospitalization, his serum creatinine levels had returned to baseline (0.8-0.9 mg/dL).

DISCUSSION

High-dose methotrexate treatment of hematologic malignancies results in AKI in 2%-12% of patients.[1] Treatment involves hydration, alkalinization of the urine, and the use of glucarpidase to metabolize the MTX and reduce crystallization in kidney tubules. Functional recovery usually occurs, although dysfunction can persist for several months in a subset of patients.[9] In our patient, given the presence of urinary tract infection, acute pyelonephritis was in the clinical differential diagnosis, and because multiple antibiotics had been administered, drug-induced acute interstitial nephritis was also a consideration. At that time, cefepime was changed to ertapenem, and the patient was treated with a course of steroids. Tumor lysis syndrome was deemed very unlikely given a low tumor burden and normal calcium and phosphate; treatment would include IV hydration, rasburicase, and possible treatment with dialysis. In the event of thrombotic microangiopathy (considered unlikely given a normal haptoglobin and lactate dehydrogenase), treatment would have been the cessation of offending agent and further etiologic work-up, such as investigation of complement pathway abnormalities and possible treatment with eculizumab. Infiltration of the kidney by lymphoma can result in tubular dysfunction and AKI; management of the patient in this situation would include treatment of the lymphoma and corticosteroid administration.

In the typical clinical setting of AKI after treatment with hd-MTX, kidney biopsy is not usually performed—AKI is presumed to be because of MTX precipitation. When biopsy is done, MTX crystals can often be identified using standard light microscopy technique on formalin-fixed, paraffin-embedded, H&E-stained sections as annular, yellowish-brown aggregates of crystalline material within tubule lumens, usually associated with morphologic evidence of tubular injury. The crystalline material shows birefringence on polarization of the light. Microscopic analysis of the urine can demonstrate the crystals as brown aggregates.[2-5]

In the current case, despite a high MTX serum level, the presence of AKI, and evidence of tubular injury on kidney biopsy—all of which are consistent with MTX-induced toxicity—MTX crystals were not seen in the urine sediment or on sections stained with standard histochemical stains for light microscopy, even with polarization of the sections. Nor were crystals identified on frozen sections obtained for immunofluorescence microscopy. Only the tissue submitted for ultrastructural examination contained the diagnostic crystals.

Several reports have described the morphology of MTX crystals on light microscopy using H&E and silver stains,[3,6-8] whereas to our knowledge, ours is the first case to describe MTX crystals on toluidine blue-stained sections prepared for TEM and their ultrastructural appearance in a kidney biopsy.

There are scarce reports on the ultrastructure of drug crystals seen in kidney biopsies. To date, only foscarnet-induced crystal nephropathy has been characterized on TEM.[10] This may be because of sampling error, missing the material, or tissue processing that mechanically removes or dissolves crystals. For example, foscarnet crystals are water soluble and are dissolved using formalin fixation; Plesko et al[10] circumvented this problem by fixing the tissue in alcohol, although some crystals may still be damaged or removed during the cutting of paraffin sections. The crystals were identified in glomeruli and tubules and were described to be rectangular and plate-like. As demonstrated in the current case, MTX crystals are not dissolved during standard processing of the tissue for TEM and also retain their characteristic birefringence on toluidine blue-stained sections using polarized light. This case emphasizes the importance of thorough review of biopsy tissue to reduce the chance of missing a diagnostic feature by sampling error.

Of further note, MTX crystals in the current case were identified in the tubules, not in the glomeruli; drug-related crystals are typically in the tubules rather than the glomeruli; however, ultrastructural examination of kidney biopsy tissue is commonly glomerulocentric. Therefore, special attention during examination for EM should also be paid to the tubulointerstitium, especially when an expected cause of kidney injury is not identified on the tissue for light or immunofluorescence microscopy. A combination of the tinctorial qualities and birefringence on light polarization of the specimen on Epon-embedded, toluidine blue-stained sections, and the ultrastructural features of MTX crystals may be useful to distinguish them from other crystals or material identified in kidney biopsy tissue.

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