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Case Report

Topical calcium folinate wet compress for treating skin damage caused by high-dose methotrexate chemotherapy: two case reports

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ABSTRACT

High-dose methotrexate (HDMTX) chemotherapy is a crucial method for consolidating the therapeutic effect of acute lymphoblastic leukemia (ALL). However, its side effects, particularly in patients with mutations in multi-drug resistance gene 1 (*ABCB1*) and/or methylene-tetrahydrofolate reductase (*MTHFR*) genes, should not be disregarded, as their abnormal MTX metabolism can lead to increased organ damage. This study reported two cases demonstrating the management of skin damage caused by HDMTX chemotherapy. A mixture of 0.9% saline (100 mL) and 100 mg of calcium folinate (CF) was prepared. CF saline was sprayed onto the skin using a disposable sterile syringe, or sterile gauze soaked in CF saline was applied as a wet compress on the affected skin areas. Ultimately, the skin damage caused by HDMTX chemotherapy in two patients was recovered within a short time. This study suggests that topical calcium folinate wet compress is convenient and effective in treating skin damage caused by HDMTX therapy, especially in patients with slow metabolism.

Keywords: calcium folinate, high-dose methotrexate, skin damage, rash

INTRODUCTION

Methotrexate (MTX), a cycle-specific chemotherapy drug, is a biologically active folate cofactor that primarily inhibits dihydrofolate reductase (DHFR). This inhibition prevents dihydrofolate from being reduced to physiologically active tetrahydrofolate, thereby blocking DNA synthesis[1]. High-dose methotrexate (HDMTX) can penetrate the blood-brain barrier and blood-testis barrier, making it a first-line therapeutic option for acute lymphoblastic leukemia (ALL), with its clinical efficacy well-documented in numerous studies[2–4]. While high-dose administration achieves therapeutic effects, it also increases the risk of various adverse reactions. Clinical studies indicate that the occurrence of adverse reactions, including myelosuppression, rash, gastrointestinal reactions, and liver function damage, is associated with elevated blood drug concentrations and delayed MTX excretion. Persistent high concentrations of MTX can lead to a significant increase in the incidence of adverse reactions[5].

The toxicity associated with HDMTX can be mitigated through intravenous administration of calcium folinate (CF), which can protect normal tissue cells from MTX-induced damage to some extent.

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However, some patients have drug metabolism gene mutations, particularly in the methylenetetrahydrofolate reductase (*MTHFR*) gene or/and the multidrug resistance gene 1 (*ABCB1*), which can lead to MTX metabolism disorders and exacerbate adverse reactions[6]. While liver and kidney function damage following HDMTX administration have been extensively documented, there were few reports of skin rashes or even severe acute exfoliative dermatitis. This study reported the successful treatment of two patients with skin damage following HDMTX chemotherapy using topical CF wet compress, which achieved favorable recovery outcomes.

CASE PRESENTATION

Case 1

This case involved a 24-year-old male patient (height: 178 cm; weight: 75 kg) who presented in August 2020 with symptoms of fatigue and dizziness. Bone marrow aspiration (BMA) revealed a lymphoblastic ratio of 93.5%, with positivity for the TEL/AML1 fusion gene (*TEL-AML1*) and Wilms' tumor-1 gene (*WT1*). The Flow Cytometry (FCM) analysis showed the blast with $CD34^{+}CD117^{-}DR^{+}CD38^{+}CD13^{dim}CD33^{+}CD123^{-}CD15^{-}CD10^{-}CD20^{-}CD19^{+}CD22^{+}CD7^{-}cCD3^{-}cCD79^{dim}MPO^{-}$. The next-generation sequencing (NGS) analysis indicated a 100% mutation in *ABCB1* at the C3435T (rs1045642) location, indicating a slow metabolic type (TT gene type). Finally, the patient was diagnosed with B-cell ALL. On September 21, 2020, induction chemotherapy was initiated with a regimen of prednisone, vincristine, idarubicin, and pegaspargase (VILP). A bone marrow retest on October 14 showed 2% of blast cells with positive Minimal Residual Disease (MRD) by FCM, and persistent *TEL-AML1* fusion gene expression. The patient subsequently received chemotherapy with the CAL regimen (cyclophosphamide, cytarabine, and pegaspargase) on October 30. A bone marrow detection on November 18 indicated morphological complete response (CR) with negative MRD by FCM, though molecular testing remained positive for *TEL-AML1*. On December 2, the patient was administered 3.5 g of MTX on day 1 and 1.0 g of cytarabine every 12 hours from day 2 to day 5 (MA regimen). Twelve hours after chemotherapy completion, routine intramuscular CF was performed for detoxification.

On the 5th day of chemotherapy, the patient developed a red rash on both upper extremities, accompanied by pruritus. Symptomatic treatment with antipruritic and corticosteroid creams proved ineffective. By the 3rd day post-chemotherapy, the rash had progressed to involve the abdomen and back, accompanied by epidermal desquamation, oral ulcers, and elevated creatinine levels. These symptoms were considered adverse reactions to MTX, but the test of MTX concentration at 72 hours was 0.06 μmol/L (≤ 0.1 μmol/L), meeting the detoxification criteria. Thus, further intramuscular injection of calcium leucovorin for detoxification was not administered. Concurrently, the patient developed a fever of 39 ℃. The blood count test revealed a white blood cell (WBC) count of 1.65×10^{9}/L, a neutrophil (NEUT) count of 1.58×10^{9}/L, an hemoglobin (HGB) level of 84 g/L, and a platelet (PLT) count of 113×10^{9}/L. The fever was attributed to neutropenia with infection. Given the damage to the skin and mucosal barrier that could exacerbate the infection, empirical treatment with imipenem-cilastatin and vancomycin was administered for anti-infection. For rash management, a mixture of 0.9% saline (100 mL) and CF (100 mg) was prepared. The affected skin areas were treated by spraying CF saline using a disposable sterile syringe or by applying sterile gauze soaked in CF saline as a wet compress. After one week of treatment, the rash significantly improved and fully resolved after one month (***Fig. 1***). Subsequently, the patient received an allogeneic hematopoietic stem cell transplantation (allo-HSCT) from an unrelated donor with a human leukocyte antigen (HLA) 9/10 match. The patient remains alive at follow-up.

Case 2

A 34-year-old female patient (height: 162 cm; weight: 47.5 kg) presented with abdominal pain in March 2021. BMA revealed a proportion of primitive lymphocytes of 91%, with an ataxia telangiectasia-mutated gene (*ATM*) of 48.4%. The FCM analysis showed that the tumor blasts expressed CD34, CD19, CD10, HLA-DR, CD33, and CD45. There was a homozygous mutation at position 677 of *MTHFR* gene, with a genotype of T677T. The patient was diagnosed with common B-ALL with myeloid expression. The patient underwent three courses of chemotherapy at a local hospital: one course of the VDLD regimen (vincristine, daunorubicin, pegaspargase, and dexamethasone) and two courses of CAML regimen (cyclophosphamide, cytarabine, 6-mercaptopurine, and pegaspargase) for intensification. On August 5, 2021, a bone marrow detection indicated CR with negative MRD by FCM. However, the patient experienced severe bilateral hip pain

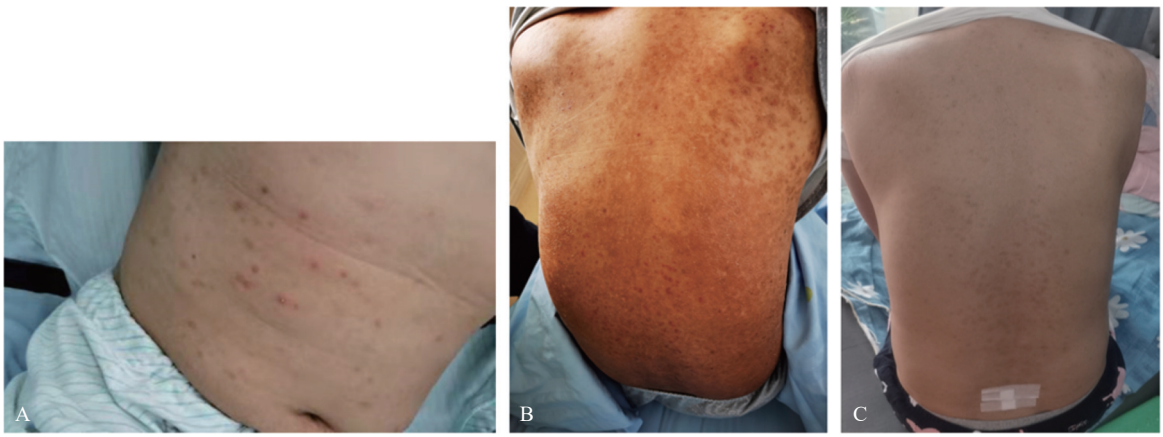

***Fig. 1* Rash condition of the first patient.** A: The first day of the rash; B: One week after rash appeared; C: Recovered after one month.

during this period. Magnetic resonance imaging (MRI) revealed multiple abnormal signals in the upper parts of bilateral femurs, bilateral iliac bones, right acetabulum, ischium, and pubic symphysis, which suggested potential leukemia infiltration. Given the indications for allo-HSCT, the patients was referred to our hospital for treatment. The patient had not previously received HDMTX consolidation therapy, presumably because the local doctors were concerned about her *MTHFR* gene mutation and the risk of organ failure associated with HDMTX. However, due to multiple bone lesions in this instance, during the preparation for stem cell transplantation, we administered MA intensification therapy (methotrexate 1.5 g on day 1, followed by cytarabine 1.5 g every 2 hours from day 2 to day 3) on August 15. Considering the *MTHFR* gene mutation, the MTX dose was reduced by two-thirds. A conventional CF intramuscular injection was administered for detoxification 12 hours post-chemotherapy, while monitoring the MTX concentration to reach the target range.

Seven days after MTX chemotherapy (August 23, 2021), the patient developed skin pruritus on the neck, waist, and bilateral upper extremities, with a patchy rash appearing. Blood count test indicated a WBC count of 2.2×10^9/L, a NEUT count of 1.9×10^9/L, an HGB level of 64 g/L, a PLT count of 84×10^9/L, a creatinine level of 67.5 μmol/L, a urea level of 3.28 mmol/L, and a normal glomerular filtration rate of 102 mL/min. However, liver function tests showed significantly lifted transaminases (ALT 132.3 IU/L, AST 32.5 IU/L, and GGT 205.4 IU/L), prompting initiation of drug therapies for liver protection and enzyme reduction. As therapeutic MTX levels had not yet been achieved, CF intramuscular injection was continued for detoxification. Notably, the patient's MTX concentrations remained above the standard limit at all measured time points after administration: 1.42 μmol/L on day 2, 0.7 μmol/L on day 3, 0.3 μmol/L on day 4, and 0.04 μmol/L on day 9. For the rash, a mixture of 0.9% saline (100 mL) and CF (100 mg) was prepared, and sterile gauze soaked in this solution was applied as a wet compress to the rash areas. This intervention resulted in significant clinical improvement within 3 days, with a marked reduction in rash and resolution of symptoms such as pruritus (***Fig. 2***). Following this treatment course, the patient's bone pain symptoms improved significantly. Subsequently, the patient received an allo-HSCT from her sister with an HLA match of 6/12. The patient remains alive at last follow-up.

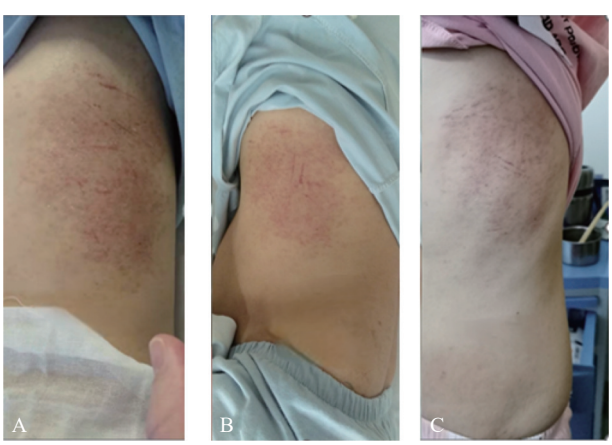

***Fig. 2* Rash condition of the second patient.** A: Day 8 after MTX; B: Day 1 after calcium folinate wet compress; C: Day 3 after calcium folinate wet compress.

DISCUSSION

HDMTX significantly increases drug con-

centrations in both blood and cerebrospinal fluid, playing an important role in improving remission rates for leukemia and other tumors. However, this treatment is associated with notable toxicity. Impairment of liver and kidney function, as well as mucosal damage, are the most frequently observed adverse reactions associated with high-dose methotrexate. Among these, mucosal damage represents the most common complication of MTX chemotherapy, typically occurring within 3 to 7 days after administration. Skin symptoms primarily manifest as erythema, dry desquamation, pruritus, etc[7]. Currently, there are several protocols exist for managing liver and kidney damage caused by MTX, including administering hepatoprotective and enzyme-lowering drugs, hydrating and alkalizing the body, detoxifying with calcium leucovorin, and in severe cases, performing dialysis. In contrast, MTX-associated skin damage remains a therapeutic challenge, which is often overlooked when mild or difficult to manage when severe. In the two cases presented in this study, skin damage occurred following MTX chemotherapy. Conventional symptomatic treatment with standard medications yielded poor results. Fortunately, applying calcium leucovorin as an external wet compress resulted in significant and rapid improvement. To our knowledge, this is the first documented report of this result.

According to the World Health Organization (WHO) grading criteria for anticancer drug toxicities and side effects, skin symptoms associated with HDMTX are classified as follows: Grade 0 (asymptomatic), Grade 1 (erythema), Grade 2 (dry dermatitis and pruritus), Grade 3 (wet dermatitis), and Grade 4 (exfoliative dermatitis)[8–9]. Case 1 in our report exhibited Grade 4 severe toxicities and side effects, including exfoliative dermatitis, as well as severe toxicities in the mouth and digestive system. Patient 2 presented with Grade 2 skin toxicity characterized by erythema and a red acne-like rash with pruritus. Both patients experienced mild kidney dysfunction that recovered after symptomatic treatment. Each received leucovorin detoxification therapy promptly within 12 hours after the completion of MTX chemotherapy. Simultaneously, MTX concentrations were closely monitored, and the frequency of CF rescue was extended if the concentrations remained high. MTX-induced skin adverse reactions are rare but serious. The skin toxicological histopathological features induced by MTX involve keratinocyte swelling, dyskeratosis, and epidermal necrosis, frequently accompanied by lymphocyte and eosinophil infiltration[10]. The skin and mucosal adverse reactions caused by MTX often occur before bone marrow suppression, serving as indicators of other MTX adverse effects[11]. Therefore, it is essential to closely monitor MTX blood concentrations, providing a basis for detoxification treatment.

Both patients reported in this study exhibited gene mutations related to drug metabolism. Case 1 presented with an *ABCB1* mutation. ABCB1 (also known as multiple drug resistance 1 or P-gp) is an ATP-binding cassette (ABC) transporter protein encoded by the *ABCB1* gene in humans[12]. To date, 48 single nucleotide polymorphisms (SNPs) have been identified in the *ABCB1* gene, among which the C-to-T mutation at position 3435 (rs1045642) in exon 26 is a synonymous mutation. Although the amino acid sequence remains unchanged, the protein structure may be altered, affecting its function[13]. The ABC transporter family is a large class of transmembrane proteins involved in the absorption, transport, and excretion of various substances. The efflux of MTX from cells is mediated by members of the ABC transporter family. Studies showed that *ABCB1*, *ABCC1-4*, and *ABCG2* play important roles in MTX efflux[14] . Currently, the most extensively researched gene is *ABCB1*, which encodes P-glycoprotein (P-gp) and participates in MTX efflux-related transport[15].

The development of rash in Case 1, despite MTX blood concentrations within the normal metabolic range, may be explained by several potential mechanisms related to genetic variation (*ABCB1* C3435T mutation) and tissue-specific factors. A detailed analysis is as follows: (1) *ABCB1* mutation and MTX transport. The *ABCB1* gene encodes P-gp, a "drug efflux pump" that transports drugs from cells (e.g., skin cells) into the bloodstream for elimination. The C3435T mutation reduces P-gp activity, causing MTX to "accumulate" in tissues like the skin. Even with normal blood MTX levels, local skin accumulation due to impaired efflux can induce direct cell damage or inflammatory reactions (e.g., rashes). (2) Skin-specific pharmacokinetics. Compared to blood, the skin has lower blood flow and reduced metabolic enzyme activity, resulting in delayed drug clearance. Even with adequate blood levels, the drug may persist in skin tissue, leading to toxicity. MTX works by inhibiting folate metabolism (blocking dihydrofolate reductase, DHFR). Keratinocytes in the skin divide rapidly and are highly sensitive to folate deficiency. Even with normal blood drug levels, local

folate depletion can trigger inflammation and rashes. Notably, the low-dose MTX can induce skin damage[16–17].

Case 2 presented with a mutation in the *MTHFR* gene which is located on chromosome 1p36.3. MTHFR is a key enzyme in folate metabolism, catalyzing the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. It plays a regulatory role in DNA synthesis, activation, and repair[18]. The defects in MTHFR lead to a decrease in 5-methyltetrahydrofolate and an accumulation of homocysteine in blood, which impairs methionine synthesis as a methyl donor and interferes with DNA damage repair. MTX, as a folate reductase inhibitor, mainly inhibits dihydrofolate reductase, preventing dihydrofolate from being reduced to physiologically active tetrahydrofolate (THF)[19]. This blocks the transfer of one-carbon groups during the biosynthesis of purine and pyrimidine nucleotides, inhibiting the biosynthesis of tumor cell DNA. Therefore, the functional defects in MTHFR synergize with MTX, both increasing DNA damage and reducing repair, but also increasing the risk of adverse reactions after MTX administration[20]. The most extensively studied polymorphisms of the *MTHFR* gene are C677T and A1298C, which can decrease MTHFR enzyme activity, and variant genotypes may alter the chemotherapeutic response to folate antagonists[21]. The 677 site consists of three genotypes: wild-type CC, heterozygous mutant CT, and homozygous mutant TT. The *MTHFR* C677T gene mutation significantly affects the catalytic activity of the enzyme. The homozygous mutant genotype (TT) exhibits only 30% of the enzymatic activity observed in the homozygous wild-type genotype (CC)[22]. Patient 2 in this report had the homozygous mutant TT genotype. Therefore, the MTX dosage was reduced to one-third of the full dose, resulting in clinical symptom relief of bone pain and mild side effects such as rash. This indicates that a reduced MTX dosage is a feasible treatment approach. Current research across various fields including hematological diseases and rheumatic diseases, has shown that the toxicity and side effects of HDMTX therapy may be influenced by polymorphisms in genes such as methylenetetrahydrofolate dehydrogenase 1 (*MTHFD1*), thymidylate synthase (*TS*), γ-glutamyl hydrolase (*γ-GH*), and solute carrier family 19, member 1 (*SLC19A1*)[23–26]. These genes may synergize and interact, ultimately leading to increased toxicity and side effects.

We have summarized the rationale of the wet compress method: (1) Mechanistic compatibility: Calcium folinate, as the active form of tetrahydrofolate, directly bypasses dihydrofolate reductase blocked by methotrexate, restoring intracellular folate metabolism and reducing toxicity. The local wet compress delivers THF directly to the skin, replenishing cellular folate levels, repairing damaged cells, and alleviating inflammatory responses. (2) Pharmacokinetic rationality: MTX-induced local skin toxicity may arise due to drug accumulation or metabolic variations. While systemic folinic acid rescue is effective, it may fail to achieve sufficient local drug concentrations. Wet compress enhances transdermal absorption of calcium folinate, directly neutralizing methotrexate toxicity at the site of action while minimizing systemic exposure. (3) Anti-inflammatory and restorative effects: Folate participates in cellular repair and DNA synthesis. Topical application of folate may promote skin barrier recovery, inhibit inflammatory mediator release, and indirectly mitigate rash severity. The innovative advantages of the protocol can be summarized as follows: (1) Targeted delivery for precision therapy: Conventional systemic rescue therapies may inadequately address local toxicity due to dose limitations or uneven drug distribution. The wet compress delivers the drug directly to the lesion, elevating therapeutic concentrations in target tissues, enhancing efficacy, and reducing systemic side effects (e.g., myelosuppression risk). (2) Reduced systemic burden: The local administration avoids increasing systemic folinic acid doses to lower metabolic strain, which can be particularly beneficial for patients requiring long-term or repeated MTX therapy. (3) Simplicity and cost-effectiveness: The wet compress is non-invasive, improves patient compliance, requires no complicated equipment, and can be scaled clinically.

When it comes to the limitations of this report, while various topical treatment options are available for MTX-induced skin lesions, our current evidence cannot establish calcium folinate as the most efficacious option. This would require randomized controlled studies. Additionally, no pathological evidence of the skin was retained when these two patients developed rashes, which is another limitation of this report.

We proposed the following directions for future research: development of transdermal formulations (such as gels or patches) that enhance absorption through specialized forms like liposomes or nanocarriers; incorporation of moisturizers (such as

petroleum jelly) or low-potency corticosteroids to potentially enhance protective effects; and monitoring of MTX blood concentrations to avoid excessive accumulation.

Author contributions

Conceptualization, Xiaoli Chen and Yimei Feng; Methodology, Li Wang, Xiaoqin Zhou, Hengrui Sun, and Yimei Feng; Writing – Original Draft Preparation, Hengrui Sun, Xiaoqin Zhou, Jie Tang, and Jia Liu; Writing – Review & Editing, Xiaoli Chen and Yimei Feng; Supervision, Xi Zhang; Project Administration, Li Wang, Feng Ran, Jie Tang, and Yimei Feng; Funding Acquisition, Xiaoli Chen, Yimei Feng, and Xi Zhang.

Ethics statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and was approved by the Ethics Committee of the Second Affiliated Hospital of Army Medical University.

Informed consent statement

The patients/participants provided written informed consent for the publication of this case, including the publication of images.

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Declaration of competing interest

The authors declared no conflict of interests.

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