Role of folinic acid in methotrexate-based prophylaxis of graft-versus-host disease following hematopoietic stem cell transplantation

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ABSTRACT

Methotrexate (MTX) is one of the main therapeutic agents currently used for the prophylaxis of graft-versus-host disease (GvHD) following hematopoietic stem cell transplantation. However, it is associated with significant toxicity and considerable side effects in many patients, which lead to either early withdrawal or dose reductions that may expose patients to the risk of GvHD and graft failure. Folinic acid (FA) can bypass the inhibitory effects of MTX on folate availability and control MTX toxicity. However, concerns that FA might inhibit the anti-GvHD effect of MTX and limited reports on its clinical usefulness have led to reluctance in its inclusion in standard GvHD prophylaxis regimens. Additionally, universal dosing and timing guidelines are lacking. I discuss the available literature and evaluate the evidence for the effect of FA on MTX toxicity and its safety regarding GvHD development and graft rejection in both adult and pediatric patients. Although FA administration appears to be safe, its efficacy for routine use in all types of transplants in adult patients is unproven and further research is required to confirm its MTX toxicity-lowering effect, identify the individual parameters that influence its usefulness in clinical practice, and evaluate its potential when developing a personalized prophylaxis regimen.

Introduction

The field of bone marrow transplantation (BMT) was pioneered by E.D. Thomas, who performed the first human allogeneic BMT in 1957 [1]. His continued work in the field was instrumental in identifying graft-versus-host disease (GvHD) as a major factor in transplant failure and for developing methods to identify and type human leukocyte antigens (HLA) [2]. Based on animal and preliminary human studies, Thomas et al. reported the first use of methotrexate (MTX) for the prevention of GvHD in a study with HLA-matched donor-recipient sibling pairs in 1971 [3]. Although the introduction of MTX prophylaxis was found to successfully prevent the development of GvHD in several patients, Thomas et al. recognized that MTX is associated with significant toxicity, including the development of mucositis [3]. Today, MTX is well recognized to cause widespread toxicity in many patients, affecting organs such as the skin, gut, liver, and kidneys, and induce delayed hematopoiesis [4,5]. Interestingly, Thomas et al. asserted that sensitivity to MTX is related to folate deficiency, and treatment with this drug can be resumed once dietary intake of folate is restored [3]. Studies have demonstrated that MTX prophylaxis prolongs survival following allogeneic BMT in mice and dogs; therefore, MTX was introduced as a standard immunosuppressive agent following BMT in humans [6]. However, by the end of the 1970s, it became clear that, in practice, MTX dosing often had to be withheld or modified because of toxicity issues [6].

Today, GvHD remains a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). Some patients omit the last dose of MTX on day 11 because of mucositis or other organ toxicity, which results in a higher non-relapse mortality rate, although the higher mortality rate could be a result of preexisting severe mucositis that is associated with lower survival [7]. Several pharmacological strategies are available for GvHD prophylaxis. In addition to MTX, other available options include calcineurin inhibitors (such as cyclosporine A [CsA]), macrocytic lactones (such as tacrolimus and sirolimus), corticosteroids, and mycophenolate mofetil. Although their mechanisms of action differ, the overall aim of these prophylactic drugs is to induce immunosuppression; hence, off-target effects and toxicity are inevitable, regardless of the treatment strategy selected [8]. To counteract MTX toxicity, Gratwohl et al. added the known MTX antidote, folic acid (FA), to the MTX prophylaxis regimen in their study in dogs [6]. Encouragingly, their results demonstrated that FA significantly reduces MTX toxicity. In 1989, the work was continued by Torres
et al., who performed the first human study of MTX with FA rescue as GvHD prophylaxis [9]. These researchers compared CsA and MTX + FA for GvHD prophylaxis. They found that both treatment regimens prevent GvHD to a similar extent; however, patients treated with MTX + FA showed reduced renal and hepatic toxicity (Table 1) [9]. In this review, I discuss the available literature and evaluate evidence for the effect of FA on MTX toxicity and its safety for GvHD development and graft rejection in adult and pediatric patients.

**Relationship between MTX and FA**

MTX is an antimetabolite and antagonist of folic acid. Folic acid acts as a cofactor for the methyltransferases involved in thymidine and purine biosynthesis, such as dihydrofolate reductase (DHFR). Thus, MTX administration inhibits cellular processes such as DNA and RNA synthesis, protein synthesis, and cell division, with dramatic consequences for the generation of a successful immune response. The suppression of T-cell responses and proliferation and the expression of adhesion molecules is critical for successful MTX immunosuppression [19]. Although anti-inflammatory and antiproliferative effects are crucial for the ability of MTX to mediate immunosuppression to prevent GvHD, these effects, unfortunately, cause significant toxicity and side effects in many patients [20].

Studies have shown that MTX administration post-transplant may increase the risk of oral mucositis, delayed engraftment, hepatotoxicity, and nephrotoxicity, which are associated with higher morbidity and mortality, especially among patients in high-risk groups [11,17,18,21,22]. Therefore, physicians may omit or reduce the MTX dose to avoid such complications, typically if the patient has existing severe mucositis, renal or hepatic impairment, or third spacing, which may increase their risk of developing GvHD or graft failure [5]. In particular, the development of mucositis has been a difficult challenge following MTX administration as it is associated with substantial morbidity, contributes to transplant-related mortality, and has proven largely resistant to most efforts that aim to reduce its incidence and severity [13].

FA is a derivative of tetrahydrofolic acid and can be readily converted to other folic acid derivatives. It is produced via the conversion of dihydrofolic acid, which requires DHFR activity. In contrast to tetrahydrofolic acid, the conversion of FA to folic acid can proceed independently of DHFR; thus, the process is unaffected by MTX treatment. FA can also reactivate DHFR and restore its activity, even in the presence of MTX [12]. FA, therefore, allows purine and pyrimidine synthesis to occur to a certain extent in the presence of MTX. Additionally, low-level DNA replication can occur, thereby limiting the toxic effects of MTX.

**Clinical use of FA**

In 2000, the European Group for Blood and Bone Marrow Transplantation performed a survey on FA use in its transplant centers (Table 1). Overall, they found that most responders agree that FA should be administered after MTX to avoid side effects, such as myelosuppression and mucosal damage. The accepted dose of FA among the surveyed centers was 15 mg/m²/day initiated 24 h after the first MTX administration [16]. However, the survey also confirmed that, in practice, less than 50% of the responding centers routinely use FA to reduce MTX toxicity [10]. Comparable numbers were reported by Bhurani et al. who performed a similar survey in transplant centers in Australia and New Zealand in 2008 (Table 1) [13]. These researchers also highlighted the difference when treating adult and pediatric patients; only 33% of the surveyed centers used FA rescue following adult transplants, whereas 67% used it routinely after pediatric procedures [13]. These responses indicated that the usefulness of FA remains controversial among clinicians. Additionally, concerns that FA might abrogate the anti-GvHD effect of MTX have led to a lack of consensus regarding the use of FA and, nearly 50 years after Thomas’ pioneering ventures into BMTs, no clear guideline or evidence exists for the timing and dosing schedule of FA [10,13].

**Effect of FA on MTX toxicity**

In 2012, Sugita et al. conducted a study in 118 adult patients administered GvHD prophylaxis with either MTX + CsA or MTX + tacrolimus (Table 1). Twenty-nine patients received systemic FA at doses corresponding to their MTX dose every 6 h, starting 12 h following the first dose of MTX [23]. They observed a significant reduction in the incidence of oral mucositis in patients receiving FA rescue compared with those who did not. Freyer et al. presented corroborating results from a study in 65 adult patients on a similar dosing regimen with either MTX alone or MTX in combination with FA (Table 1) [14,24]. Although statistical significance was not reached for the lower incidence of mucositis, a shorter overall duration of mucositis was confirmed in patients who received FA. Limitations to the retrospective studies include their retrospective nature, different intensities of conditioning chemotherapies used, different dosing schedules, and that mucositis severity was assessed using different scales, including the National Cancer Institute Common Terminology Criteria for Adverse Events, Oral Mucositis Rating Scale, and Oral Mucositis Index. Recently, Yeshuran et al. [25] performed a randomized,
| Reference | Type of BMT | Conditioning intensity | Patient group | FA regimen | GvHD prophylaxis | Outcomes |
|-----------|-------------|-----------------------|---------------|------------|-------------------|----------|
| [10]      | Full HLA MRD MAC/RIC | 140 adult patients | 10 mg/m² IV q.6 h × 4 doses in total, commencing 24 h post-MTX | Standard MTX 33% vs. 39% prophylactic corticosteroids | No significant association between FA and mucositis grade or duration of high-grade mucositis | No FA → earlier + more severe GvHD in patients who received a 677CC transplant |
|           |             |                       |               |            | FA → longer total duration of mucositis, especially with 677CT transplant | FA → decreased survival and EFS in patients administered a 1298AC transplant |
|           |             |                       |               |            | No FA → earlier + more severe GvHD in patients who received a 677CC transplant | FA → increased survival in patients receiving 677CC transplant |
| [11]      | MUD-BM/PB or MUD-BM/PB MAC/RIC | 118 adult patients | FA administered 12, 18, and 24 h after MTX on D1 and D3; 24, 30, and 36 h after MTX on D6 (29 patients) | FA mouthwash (13%) 4× daily D1-D7 | Oral mucositis incidence significantly reduced (IV FA) | No change in acute GvHD incidence on D100 |
|           |             |                       |               |            | Tendency to reduce oral mucositis with FA mouthwash | No significant difference in non-relapse mortality |
| [12]      |60% MUD MAC | 65 adult patients | FA (15 mg) administered PO q.6 h × 4 doses, 12 h post-MTX on D3, D6, and D11 | MTX + FA | Shorter duration | Unchanged |
|           | (Abstract) |                       |               |            | NR | Shorter time to neutrophil engraftment |
| [13]      |68% MUD in FA Related | 51 adult patients | 15 mg PO q.6 h × 4 doses 12 h post-MTX on D3, D6, and D11 | MTX + FA | Shorter duration | Unchanged |
|           | Group 62% MUD in control |                       |               |            | NR | Unchanged |
|           | Related |                       |               |            | NR | Unchanged |
| [14]      | NR in abstract Related | 32 adult patients NR in abstract | 5 mg q.6 h starting 24 h post-MTX and continuing until 6 h before the next MTX dose | MTX + CsA Broad-spectrum antibiotics | Lower incidence of regimen-related toxicity (somatic/hepatic) | No difference in Grade II-IV GvHD incidence |
|           |             |                       |               |            | No difference in chronic GvHD prevention by MTX | No difference in chronic GvHD prevention by MTX |
| [15]      | NR in abstract Related | 69 adult patients NR in abstract | 5 mg q.6 h starting 24 h post-MTX and continuing until 6 h before the next MTX dose | Standard MTX + CsA | Improved tolerance to MTX, all doses given | No effect on GvHD prevention by MTX |
|           |             |                       |               |            | No difference in OS at 9 months FU |
| [16]      | MSD MAC | 57 adult patients 1:1 with MTX, 24 h post-MTX | 26 CsA 31 MTX + FA Standard MTX | NR | No increase in GvHD with MTX + FA compared to CsA | No significant difference in rate of acute or chronic GvHD |
|           |             |                       |               |            | Reduced renal/ hepatic toxicity with MTX + FA. | No significant difference in OS at 9 months FU |
| [16]      |29% MSD in FA group 37% MSD in control group Remaining % MUD | 52 adult patients | 15 mg q.i.d., started 24 h post-MTX | Standard MTX + CsA + ATG for MUDs | No significant difference in rate, duration, or severity with FA compared to placebo | No significant difference in rate of acute or chronic GvHD |
|           |             |                       |               |            | No significant difference in OS at 9 months FU | NR |
placebo-controlled study in 52 adult patients who had a BMT and received either FA or a placebo in addition to MTX + CsA prophylaxis. FA or placebo administration started 24 h after each MTX dose and was given during 24 h: 15 mg three times daily after MTX administration on post-transplantation day +1 and four times daily after MTX administration on days +3 and +6. No improvement in the rate of grade 3–4 oral mucositis or secondary outcomes, which included engraftment, the need for IV opioids, and transplant-related mortality and survival, could be identified in the FA group at the interim analysis, and the study was discontinued early. Important to note that this study did not include pediatric patients and FA was initiated at 24 h after MTX administration, whereas in the three positive studies, FA was started earlier (12 h after MTX administration). Two other significant retrospective studies, involving 311 and 140 adult patients, respectively, indicated that, compared with MTX alone (Table 1), combined treatment with MTX and FA has no effect on mucositis severity [25].

One interesting aspect of transplant medicine is the possibility of tailoring medication to suit an individual. The prevalence of methylenetetrahydrofolate reductase (MTHFR) polymorphisms is estimated to be approximately 0.2% to 0.5%, although it varies by region. As reported by Murphy et al. [15], genetic analysis might serve as a useful tool for identifying patients who are eligible for FA rescue. By comparing single nucleotide polymorphisms (SNPs) in MTHFR in donors and recipients, patients who received a transplant with the 677CT SNP were recognized to have increased susceptibility to a longer total duration of mucositis following FA administration (Table 1). As further discussed below, transplant MTHFR SNPs affect the response to FA via several mechanisms that are worth considering before the administration of FA.

Reports on the effect of FA on mucositis in pediatric patients are scarce. Kodama et al. [26] reported a study that included 111 pediatric patients in 2015. Patients received either MTX + CsA or MTX + tacrolimus as GvHD prophylaxis, and 61 of them received high- or low-dose FA in addition to GvHD prophylaxis (Table 2). Comparison of the treatment groups showed no significant difference in mucositis severity. In this study, mucositis severity was evaluated based on duration, trismus, inability to eat, and opioid administration; however, this evaluation method was found not relevant to the actual mucositis severity.
to deviate from the scales used in studies with adult patients. Hudspeth et al. used the delivery of the fourth dose of MTX as a surrogate for MTX toxicity in 47 patients who were included in their study because of inter-patient variability in toxicity grading, including that in mucositis [27]. Of the patients who received FA in addition to MTX + CsA prophylaxis, 98% received the MTX dose on day 11. Only 48% of patients who did not receive the FA supplement could tolerate the day 11 MTX dose because of toxicity, including mucositis (Table 2).

**Patient safety and other outcomes of FA administration**

One of the main reasons for the lack of FA administration, despite its potential ability to limit the incidence of mucositis, is the concern that it might counteract the anti-GvHD effect of MTX [10,13]. There currently is no evidence of an increase in the risk of GvHD in either adult or pediatric patients treated with FA. Studies enrolling a total of approximately 800 adult patients suggest that the risk of acute GvHD remains unchanged in patients who receive FA rescue compared with those receiving MTX + CsA/tacrolimus prophylaxis alone (Table 1) [8,23,25,27,28].

In studies in pediatric patients, both Hudspeth et al. and Lindqvist et al. compared the risk of high-grade GvHD between patients treated with FA and MTX + CsA/tacrolimus and those treated with MTX + CsA/tacrolimus alone. Similarly, they did not identify an increase in the risk of high-grade GvHD in patients treated with FA (Table 2) [26,29]. These findings support the safety of FA when combined with MTX.

Another important outcome associated with GvHD is survival. Assessment of the survival outcomes in studies in adult patients has been sporadic (Table 1). Nevill et al. performed a small sample size study involving 32 adults who received MTX + CsA as GvHD prophylaxis. Nonetheless, the researchers could not identify any difference in event-free survival between patients who received FA rescue and those who did not [28]. This finding was supported by the findings of Sugita et al. in 2012, who confirmed that there is no significant difference in non-relapse mortality following GvHD regardless of whether the patients received FA [23]. Similarly, in an interim analysis, Yes-hurun et al. found no significant difference in overall survival 9 months after BMT between adult patients who received FA rescue and patients who received a placebo [25]. One exception that warrants caution is the presence of certain SNPs in MTHFR, as discussed above. Encouragingly, Murphy et al. confirmed that FA treatment prolongs the survival of patients who receive a 677CC transplant. However, they also noted a shortened survival in patients who received a 1298AC or 677CT transplant and were subsequently treated with FA. This finding further supports the requirement for the inclusion of MTHFR genotypes in the selection of certain donors, if deemed suitable and applicable, to individually determine the suitability of FA addition and improve patient outcomes [15].

Kodama et al. assessed 3-year survival after allogeneic HSCT in 111 pediatric patients; no difference was found between patients who received FA with GvHD prophylaxis and those who did not receive this regimen [26]. Hudspeth et al. also observed no difference in relapse-free survival in the 47 pediatric patients included in their study [27]. Overall, these findings were supported by the findings of Lindqvist et al., who found no association between FA administration and disease relapse [29]. Of note, this study used a different FA administration schedule and lower FA dose of 10 mg/m² that was started the day before the first MTX administration (Table 2) [30].

Additional factors to consider during the post-transplant period include time to engraftment and the potential of graft rejection. Several studies have confirmed that adding FA to MTX prophylaxis shortens the time to neutrophil engraftment after HSCT [14,15,27]. Notably, in pediatric patients, high-dose FA (25 FA doses, compared to 4 doses in the low-dose group) is required to reduce the neutrophil engraftment time, suggesting that the response may be dose-dependent (Table 2) [26]. Nonetheless, it is worth highlighting that evidence for graft rejection is conflicting. In fact, no association between FA treatment and graft rejection frequency has been reported for adult patients (Table 1), and reports for this association in pediatric patients are contradictory (Table 2). In line with the findings in adults, Kodama et al. confirmed that, in pediatric patients, there is no increased risk of graft rejection following FA treatment with 15 mg/m² for up to 25 doses until day 11 [26]. However, Lindqvist et al. observed an increased frequency of graft rejection in patients who were pre-treated with reduced-intensity conditioning (RIC) and received higher total doses of FA [29]. They advised that caution be exercised when adding FA to the prophylaxis regimen of RIC patients. As previously mentioned, Lindqvist et al. employed a lower dose of FA than those used in other studies (10 mg/m²) and began FA treatment before the first MTX administration, as opposed to the inclusion of a 12–24 h delay. Together with the finding that patients who receive FA starting on day 15 or later do not display an increase in the frequency of graft rejection, several interesting questions regarding FA pharmacodynamics have been raised that warrant further examination of FA timing and dosing for optimization of the treatment response.

HSCT among patients with telomeropathies is typically associated with an increased risk of transplant-related complications, including organ dysfunction,
| Reference | Type of BMT | Conditioning Intensity | Patient group | FA dose | GvHD prophylaxis | Outcomes |
|-----------|-------------|------------------------|---------------|---------|------------------|----------|
| [23]      | 23 MRD 88 MUD | MAC | 111 pediatric patients | LD-FA: 4 × 15 mg/m\(^2\) IV, 24 h post-MTX, HD-FA: MTX1: 3 × 15 mg/m\(^2\) IV starting 12 h post-MTX, MTX2: 6 × 10 mg/m\(^2\) IV starting 12 h post-MTX, MTX 3 + 4: 8 × 10 mg/m\(^2\) IV starting 24 h post-MTX | Standard MTX + FK, Standard MTX + CsA | No difference in stomatitis severity, Peak ALT lower with HD-FA, No difference in 3-year survival, Shorter time to neutrophil engraftment with HD-FA compared to LD-FA, No association with graft rejection |
| [24]      | MUD/MRD/MMU | MAC | 47 pediatric patients | 15 mg/m\(^2\) IV 12 h post-MTX | Standard MTX + CsA | Increased likelihood of receiving day +11 MTX (94% vs 48%), No effect on Grade III-IV GvHD risk in all MMU-BM patients, No effect on RFS, NR |
| [25]      | 27 HLA-MRD, 41 MUD, 19 MM | MAC (sub-study of RIC) | 87 pediatric patients | 10 mg/m\(^2\) 1: Daily from D −1 2: D −1, +2, +4, +7, +9, +12, and then daily 3: Late − starting D +15 | MTX + CsA (53%), Also, CsA + prednisolone, FK + sirolimus, and other combinations | NR, No correlation between FA administration and GvHD ≥ Grade III, Late administration of FA had no impact on GvHD, No association between FA and relapse, Higher FA dose until D +21 → graft rejection, RIC patients: higher FA dose → graft rejection, Late administration of FA had no impact on rejection |
| [22]      | NA | NA | Pediatric centers | FA daily, starting 24 h post-MTX | NA | Report on GvHD prophylaxis strategies |

**Abbreviations:**
- Standard MTX = 15 mg/m\(^2\) on D1 and 10 mg/m\(^2\) on D3, D6, and D11
- Type of BMT: MSD (matched sibling donor), MRD (matched related donor), MUD (matched unrelated donor), and MMU (mismatched unrelated)
- Conditioning intensity: MAC (myeloablative conditioning) vs. RIC (reduced intensity conditioning)
- NR = not reported, NA = not applicable, D = day, LD = low dose, HD = high dose, FA = folinic acid, MTX = methotrexate, EFS = event-free survival, RFS = relapse-free survival, CsA = cyclosporine A, VP = etoposide, FK = tacrolimus, TBI = total body irradiation, CY = cycarabine

**Selection Criteria:** Journal articles and conference abstracts detailing human studies of MTX in combination with FA published before March 2020 were included. Individual characteristics such as conditioning, type of BMT, MTX and FA dose, and study outcomes did not affect inclusion/exclusion.
and severe mucositis but data on FA use in this specific group, which is predominantly pediatric, are not available [31].

Conclusion

Available evidence shows that although FA can be safely administered as part of MTX-based GvHD prophylaxis for both adult and pediatric patients, its efficacy for routine use in all types of transplants is unproven based on a high-quality randomized study published by Yeshurun et al. for reducing severe mucositis; however, owing to the small number of patients included in Yeshurun et al., its conclusion cannot be generalized to pediatric patients, the majority of whom receive higher intensity conditioning chemotherapy than that used in this study. Future studies should investigate earlier administration or use different dosing regimens, which may yield positive results among certain high-risk groups for severe mucositis. Finally, caution is advised under certain circumstances, such as in patients who have received RIC or grafts with certain MTHFR SNPs or patients with telomeropathies.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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