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RESEARCH ARTICLE

Early and excessive leucovorin rescue in the prevention of toxicities after intermediate-dose methotrexate: A cross-sectional study

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ABSTRACT

Background: Due to the lack of methotrexate (MTX) therapeutic monitoring in some hospitals, clinicians often resort to fixed and higher leucovorin (LCV) doses after intermediate-dose MTX (IDMTX) infusion to prevent toxicities' occurrence. A decision that can lead to the inhibition of MTX action or even helps cancer cells to regenerate. **Aims and Objectives:** In view of the risks incurred by patients, a study was carried out to determine whether an early and excessive LCV rescue really avoids the occurrence of toxicities after IDMTX infusion and if not, identify the main encountered adverse effects by a clinical and biological monitoring. **Materials and Methods:** A cross-sectional study was conducted from November 2014 to February 2016 with a monitoring of clinical data, methotrexatemias, concentrations of biochemical parameters, and a complete blood count. Analyses were performed before the start of the infusion and 24, 48, and 72 h after. **Results:** A total of 15 patients were recruited for a total of 23 infusions of MTX at 3 g/m² over 4 h followed by an early and excessive LCV rescue, starting at the 6th h with a cumulative dose of 320 mg/m². Concentrations of MTX lower than 0.05 µmol/L were observed after 72 h in 19 infusions and after 48 h in 11. Six patients experienced toxicities with significantly higher MTX concentrations compared to patients who had no adverse effects. **Conclusion:** Despite the early and excessive LCV rescue used during the study, toxicities sometimes fatal, still took place. It is therefore recommended a methotrexatemia monitoring for at least 72 h and an evaluation of the creatinine clearance with neutrophils and lymphocytes numeration whatever the modalities of the rescue.

KEY WORDS: Intermediate-dose Methotrexate; Leucovorin; Therapeutic Drug Monitoring

INTRODUCTION

Cancer is a major public health issue where anticancer molecules represent one of the most used treatment options, allowing the inhibition of key biological mechanisms involved in the evolution of the disease.

Among these molecules, methotrexate (MTX) is an antimetabolite widely used in several chemotherapy protocols as a folic acid antagonist.[1] Its action consists in inhibiting the dihydrofolate reductase and consequently stops the production of reduced folates, essential cofactors for the synthesis of nucleotides, which induces the apoptosis of rapidly dividing cells such as cancer cells.[2]

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Although it represents a drug whose development dates from 1949,[3] it is nonetheless still responsible for numerous toxicities, mainly renal,[4-6] hepatic,[6,7] and hematopoietic[7] due to the low specificity of its mechanism of action.

The MTX prescription is therefore subject to a strict control, especially at high doses, ensuring to the patient a minimum risk of side effects that are often difficult to predict due to the patients inter- and intra-variability.[2] These precautions mainly include: A serum MTX concentrations monitoring, an alkaline hyperhydration, and a leucovorin (LCV) rescue adapted to methotrexatemias.[6]

However, it is not uncommon to note the lack of MTX therapeutic monitoring in many hospitals, particularly in developing countries. The therapy control is thus based almost exclusively on the appearance of clinical symptoms associated with toxicities and the analysis of biochemical and hemobiological parameters to confirm their manifestations.

This situation leads the clinicians to take an exaggerated preventive approach. The LCV dose, which should in principle be adjusted to methotrexatemias, is then administered at fixed doses by default. Doses that are often higher than those required. A decision that can lead to early and excessive LCV rescues supposed to prevent the occurrence of these toxicities but may instead inhibit the action of MTX or even helps cancer cells to regenerate. The risk is even more important in the case of hypersensitivity reaction with LCV.[8]

In view of the hazard incurred by patients, it was decided to carry out a study which consists in evaluating the potentially negative impact of intermediate-dose methotrexate (IDMTX) infusion in adult patients treated at the hematology department, Benflis Touhami University Hospital, Batna, Algeria and receiving an early and excessive LCV rescue.

The evaluation of adverse reactions' occurrence was based on the clinical data of each patient and the methotrexatemias measured at 24, 48, and 72 h after the beginning of the infusion. The study also relied on the variation of hemobiological and biochemical parameters' concentrations with homocysteinemia estimate. In fact, this sulfur-containing amino acid represents a potential biomarker in MTX-based therapies monitoring[9,10] and allows to check if the action of the antimetabolite took place.

The objective of the study is to determine whether an early and excessive LCV rescue really avoids the occurrence of toxicities and if not, identify the main encountered adverse effects and provide a set of recommendations to the clinicians to ensure better patient care.

MATERIALS AND METHODS

Patients and Study Design

A cross-sectional study was conducted over a period of 16 months, from November 2014 to February 2016 after approval from the Scientific Council of the Faculty of Medicine, Batna 2 University, Algeria (minutes' reference number: N°35/CSF/FM/2013), and according to the Helsinki Declaration. Patients included in the study were treated at the hematology department, Benflis Touhami University Hospital, Batna, Algeria and received IDMTX infusions followed by an LCV rescue considered early and excessive according to previous studies criteria.[11,12] Patients who received normal LCV rescue or lacked of clinical or biological data were excluded from the study. Informed consent was obtained and archived for all included subjects. For each patient, an information sheet was drawn up with the following information: Age, sex, body surface area, body mass index, main pathology, and followed protocol.

Samples and Analysis Methods

Venous blood's samples were collected in dry and heparinized tubes before the beginning of the infusion (noted 0 h) and 24, 48, and 72 h after. Hemolyzed or icteric samples were rejected. Additional samples were taken in cases of MTX elimination delays. Centrifugation was carried out immediately for 10 min at 3000 rpm. In case of analysis postponement, samples were stored at −20°C.

MTX was assayed by an enzyme immunoassay (Siemens Syva EMIT), at the Biochemistry Laboratory, Benflis Touhami University Hospital, Batna, Algeria, on the UniCel DxC 600 (Beckman Coulter, Inc., Brea, USA) with a limit of quantification of 0.05 µmol/L.[13] Biochemical monitoring included the determination of the following parameters: Urea, creatinine, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma-glutamyltransferase, and creatine phosphokinase with calculation of albuminemia before the beginning of the infusion. Analyses were performed on the dimension RxL (Siemens Healthcare Diagnostics Inc., Newark, USA). Creatinine clearance (CrCl) was estimated by the modification of diet in renal disease formula. Total plasma homocysteine was assayed on the Immulite 2000 XPi (Siemens Healthcare Diagnostics Inc., USA). The biochemical monitoring was realized at the Biochemistry Laboratory, Mustapha University Hospital, Algiers, Algeria. A complete blood count was also realized at the Hemobiological Laboratory, Benflis Touhami University Hospital, Batna, Algeria on the Medonic M32 analyzer (Boule Diagnostics, Sweden), before the beginning of the infusion and at the nadir with a numeration of leucocytes, neutrophils, lymphocytes, hemoglobin, and platelets.

Statistical Analysis

Normal distribution evaluation was carried out by the one-sample Kolmogorov–Smirnov test. Data were not normally distributed and presented as median with interquartile range. Variables comparison was carried out by the Mann–Whitney U-test and estimation of the bivariate correlation by the Spearman rank test. Statistical analyses were performed on SPSS Statistics software version 22. Statistical significance was set at $P < 0.05$.

RESULTS

At the end of recruitment, 15 patients were included with a median age of 25 years (22–29) and a male/female sex ratio of 0.87. All received a total of 23 infusions of IDMTX at 3 g/m² over 4 h and an LCV rescue that started 2 h after the completion of the IDMTX infusion, with intravenously doses of 20 mg/m² every 4 h for a total of 16 injections and a cumulative dose of 320 mg/m². Nine patients were treated for acute lymphoblastic leukemia, four for Hodgkin lymphoma, one for non-Hodgkin's lymphoma and one for acute myeloblastic leukemia. Of the 23 followed infusions, 11 presented methotrexatemias lower than 0.05 µmol/L after 48 and 8 others after 72 h.

During the study, six patients were exposed to toxicities [Table 1]. Of these patients, four had MTX elimination delays with concentrations greater than 0.05 µmol/L after 72 h, two of which had exceeded the thresholds associated with a toxicity risk,[14] with, respectively, 4.30 and 1.60 µmol/L at 72 h. Hypoalbuminemia at 18 g/L was also observed in a patient who had an MTX concentration of 15.00 µmol/L after 24 h.

Patients who experienced toxicities, also exhibited significantly higher concentrations of MTX ($P < 0.001$),

Table 1: Adverse effects encountered during IDMTX infusion

Disease	Protocol	Adverse effects	Patient status after IDMTX
Hodgkin lymphoma	IVAM[a] IDMTX[b] 3 g/m²	Headache, vomiting, rash on the face, bruising at the injection site, febrile neutropenia, and grade IV mucositis. Hypoalbuminemia at 18 g/L	Sixteen months after the IDMTX infusion, the patient died at home after several relapses and shingles development
ALL[c] B	Linker Protocol IDMTX 3 g/m²	Liver toxicity due to previously diagnosed cholestasis	The patient continued the linker protocol without other incidents
ALL T	Linker Protocol IDMTX 3 g/m²	Pancytopenia, hypothermia, and hypotension	The patient died 1 week after the infusion
Hodgkin lymphoma	IVAM IDMTX 3 g/m²	Pancytopenia	Three months after the infusion, the patient left the service without other incidents
ALL T	Linker Protocol IDMTX 3 g/m²	Disturbance of renal function (creatinemia at 504 µmol/L) with pancytopenia	The patient returned to normal status 2 weeks after infusion and left the service after completing his 9th consolidation cycle without other incidents
ALL B	Linker Protocol IDMTX 3 g/m²	Pancytopenia	Five months after the IDMTX infusion, the patient died due to aplasia and cardiac arrest

[a]Ifosfamide vincristine aracytine methotrexate, [b]Intermediate dose methotrexate, [c]Acute lymphoblastic leukemia

Table 2: Median concentrations of biochemical parameters before and after the IDMTX infusion

Parameters	Median concentrations of biochemical parameters (IQR[a])			
	0 h	24 h	48 h	72 h
Urea (mmol/L)	3.30 (2.50−0.30)	3.50 (2.70−4.80)	4.50 (3.00−5.80)	4.50 (3.00−5.80)
Creatinine (µmol/L)	44 (35−62)	53 (44−89)	62 (44−80)	62 (44−80)
Uric acid (µmol/L)	256 (200−306)	309 (196−428)	268 (235−414)	256 (208−380)
CrCl[b] (mL/min)	155.33 (113.01−241.55)	117.00 (85.94−193.24)	116.44 (89.44−171.11)	117.95 (80.74−162.58)
AST[c] (UI/L)	29.00 (20.50−38.50)	41.00 (32.00−59.00)	45.00 (28.00−65.50)	48.00 (28.00−89.00)
ALT[d] (UI/L)	18.00 (11.00−26.50)	30.00 (17.00−45.00)	28.00 (17.50−44.50)	38.00 (25.00−81.00)
ALP[e] (UI/L)	90.00 (73.50−121.50)	96.00 (79.00−114.00)	96.00 (81.50−113.00)	98.00 (73.00−123.00)
GGT[f] (UI/L)	43.00 (23.00−54.00)	39.00 (30.00−53.00)	44.00 (30.50−53.00)	47.00 (42.00−73.00)
CK[g] (UI/L)	49.00 (32.00−86.50)	53.00 (25.00−65.00)	39.00 (26.50−77.00)	41.00 (30.00−61.00)
Homocysteine (µmol/L)	7.83 (5.91−15.10)	11.70 (7.73−17.60)	8.45 (6.63−12.57)	8.10 (5.50−12.72)

[a]Interquartile range, [b]Creatinine clearance, [c]Aspartate aminotransferase, [d]Alanine aminotransferase, [e]Alkaline phosphatase, [f]Gamma glutamyltransferase, [g]Creatine phosphokinase

creatinine ($P = 0.006$), and significantly lower values of CrCl, leukocyte, and neutrophil ($P = 0.018$; $P = 0.029$; and $P = 0.039$, respectively) compared to patients who did not have adverse effects.

The median concentrations of biochemical parameters during the 72 h following the IDMTX infusion [Table 2] showed significant increases after 24 h in AST ($P = 0.018$) and ALT ($P = 0.021$), after 48 h in creatinine ($P = 0.042$) and a significant decrease in CrCl ($P = 0.036$) after 72 h compared to the values before the initiation of the MTX infusion. Methotrexatemia was also positively correlated with creatinine ($P < 0.001$), uric acid ($P < 0.001$), homocysteine ($P = 0.002$), and negatively correlated with the CrCl ($P = 0.018$). Only one patient, however, revealed a severe renal impairment, with a creatininemia up to 504 µmol/L.

Leukocytes and lymphocytes values at nadir showed a significant lowering ($P = 0.022$ and $P = 0.002$, respectively) compared to the concentrations before the beginning of the IDMTX infusion. The proportions of these decreases revealed an average decrease of 16% for neutrophils and 52% for lymphocytes.

DISCUSSION

The present study investigates the impact of IDMTX infusions in adult patients receiving an LCV rescue considered excessive, from a quantitative point of view, with a high cumulative dose of 320 mg/m² (where we speak of high dose from a cumulative dose of 315 mg/m²).[11] A rescue that is also early, with an infusion that starts at the 6th h, only 2 h after the end of the IDMTX infusion. The rescue is all the more excessive because it is systematically continued until the 70th h despite a rapid elimination of MTX in 11 of the 23 followed infusions.

Nevertheless, toxicities have still been noted in spite of these rescue modalities, with significantly higher MTX concentrations in patients with adverse effects. High values that reinforce the role of methotrexatemias as predictive markers of adverse effects' occurrence, especially the hematopoietic function disorders. The latter is the most frequently encountered in the present study, where the white blood cells seem to be the most sensitive, most likely because of the cytotoxicity action of MTX on rapidly dividing cells.

Other adverse effects are, however, uncommon with only one case of acute renal toxicity, accompanied by a significant delay in MTX elimination. A potential impact of MTX on renal function still remains where it was noted a correlation between methotrexatemia variation and CrCl values where MTX can thus be responsible for delayed elimination, causing other toxicities or aggravating them.

Regarding the liver function, transaminases' concentrations elevations are only transient, reversible and without clinical manifestation. Only a single case of acute liver toxicity is to note probably due to former cholestasis. Hypoalbuminemia seems, however, a parameter to take into account. Indeed, a methotrexatemia of 15.00 µmol/L was found at the 24th h in a patient with hypoalbuminemia of 18 g/L before the initiation of the treatment.

All of these noted toxicities and even if some are not frequent, suggest the inefficiency of the early and excessive LCV rescue to lessen the toxic effects of MTX in some patients. A finding supported by the fact that the inhibitory action of MTX appears to be occurring, with increases of homocysteinemia, 24 h after the beginning of IDMTX infusion, and 18 h after the start of the LCV rescue. A positive correlation between MTX and homocysteine's concentrations is also noted and comfort the hypothesis that the action of the MTX still takes place at this time of the infusion. In contrast, the decreases observed in homocysteinemia at the 48th and 72th h, with a return to pre-treatment concentrations, indicate that the LCV rescue becomes effective between the 24th and 48th h, with a recovery of the metabolic pathways avoiding the occurrence of potential toxicities.

When therapeutic monitoring of MTX is not available, the accentuation of LCV rescue, even in cases of IDMTX infusion, does not seem to guarantee the absence of toxicities. The adverse effects noted in the present work tend to coincide with those obtained in studies involving higher doses of MTX and accompanied by lightened LCV rescues,[5,6] especially for the renal[15] and hematopoietic functions.[16]

In fact, the case of acute nephrotoxicity found in one of the 15 patients (and equivalent to 7%) is consistent with the estimated proportions of 2–12% of the risk of developing renal disorders during high dose MTX therapy.[6] It is also important to remember that renal dysfunction is common in patients with hematological malignancies, cancers for which the MTX is often used.[17]

The results are also consistent with the findings of other studies concerning the low impact of high-dose methotrexate infusion on the liver function.[18] These studies also exclude the predictive nature of transaminases in the occurrence of toxicities. Hepatic disorders being more common during chronic oral low-dose treatments.[6]

On the other hand, albuminemia before the launch of IDMTX infusion has to be controlled, where a fall in its concentration may indeed be responsible for a slow elimination of MTX[19] as it was found in one of the followed patients.

As for the variations in homocysteine levels over the 72 h of the treatment and their correlation with methotrexatemias, these results confirm the biomarker role of this amino acid[10] and emphasize the interest it could have in case the action of MTX had to be verified, as in this study.

The data acquired in the present work draw the practitioners' attention to the still existing potential toxicity in case of IDMTX infusion and whatever the modalities of LCV rescue, even if the latter is reinforced in front of the absence of MTX therapeutic monitoring. IDMTX based therapies are also poorly studied compared to high dose MTX ones, especially when combined with early and excessive LCV rescue, which valorizes the results of this study.

However, the limited number of patients receiving IDMTX infusions with the particularity of an excessive LCV rescue remains a limitation to this work and could hide some discoveries. Further research on a larger cohort in the future would be of interest.

CONCLUSION

Clinicians' use of protocols involving early and excessive LCV rescue may be justified by the desire to ensure minimal risk to the patients, especially in the absence of methotrexatemia monitoring. It is yet recommended, in view of the occurrence of sometimes fatal toxicities during the study, to introduce therapeutic monitoring of MTX whatever the modalities of the rescue. This makes it possible to adapt effectively and objectively for each patient, the dose of LCV according to the methotrexatemias and to ensure discontinuation of the injections in the case of a fast elimination of MTX thus avoiding the rescue of cancerous cells.

For any patient receiving IDMTX infusions with or without early and excessive LCV rescue, risk of toxicity is present, hence the need to initiate MTX concentrations monitoring for at least 72 h with an evaluation of CrCl as well as neutrophils and lymphocytes numeration. Special attention should also be given to patients with albumin deficiency before the initiation of infusion.

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