

METHOTREXATE CLEARANCE AND CLINICAL TOXICITY IN OSTEOSARCOMA FOLLOWING HIGH-DOSE METHOTREXATE THERAPY

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Abstract

Two patients with osteogenic sarcoma were treated with high-dose methotrexate (MTX) followed by leucovorin 'rescue'. Profiles of MTX clearance from plasma and erythrocytes were obtained. Clearance of the drug from plasma during the first 36 hours appears to be biphasic with the first phase of elimination of the drug being appreciably more rapid than the second phase, The drug had also incorporated into the bone marrow precursor cells and reappeared after a few days in the circulating mature erythrocytes which may later serve as a slow-changing compartment for MTX. Nonspecific binding of the drug to plasma proteins may have been one of the causes of delayed clearance of plasma MTX observed in one of the patients. However, delayed clearance does not appear to correlate with the severity of clinical toxicity which was found to be more pronounced in a patient with a better clearance of the drug. Our results support the more recent concept that enhanced clinical toxicity may not be predictable by monitoring plasma MTX alone (JPMA 39: 38 ,1989).

INTRODUCTION

High dose methotrexate (0.5 g — 32.6 g) with leucovorin or citrovorum factor (CF) rescue has been widely used in the treatment of solid tumors and hematologic malignancies. The rationale behind this therapy is that the ensuing high extracellular methotrexate (MTX) concentration facilitates i) passive entry of the drug into the cells, ii) MTX polyglutamate formation, iii) entry into CNS and iv) a reduction of cellular MTX resistance¹. Due to this, the therapy is of special importance- when the tumor is MTX— resistant, such as osteosarcoma²⁻³. Moreover, therapy is beneficial in those cases as well where metastases have taken place to vital organs of the body. The risk of severe toxicity from such therapy is minimized by enhancing MTX clearance with proper hydration and alkalinization.⁴ However, if the plasma - MTX concentration remains $> 10^{-5}$ M at 24 hours or 5×10^{-7} M at 48 hours, significant toxicity is likely to occur.⁵⁻⁷ Such toxicity may be avoided by immediate intervention with leucovorin rescue.⁸ For this, routine monitoring of plasma MTX concentration is necessary to identify patients at a high risk of toxicity and to determine who will require increased doses and prolonged courses of leucovorin. Since erythrocytes have been identified as a slow changing compartment for MTX, concentration of the drug in erythrocytes should not be overlooked^{8,9}. In the present study, two patients with metastatic Osteogenic sarcoma were treated with HD—MTX therapy followed by citrovorum factor 'rescue'. Profiles of MTX clearance from plasma and erythrocytes were obtained to see if there is a correlation between clinical toxicity and levels of the drug in these two compartments.

PATIENTS AND METHODS

Clinical information about the patients is given in Table.

TABLE. Clinical Information about the two Patients.

Patient	Age	Diagnosis	Extremity involved	Chemotherapy prior to Study	Dose of MTX in this Study
G.M.	16 years	Osteogenic sarcoma with pulmonary metastasis	Right Humerus	Adriamycin and cisplatin	1st dose - 5 g /m ² (8 g), 2nd dose - 3 g/m ² (5 g)
S.H.	20 years	Osteogenic sarcoma with pulmonary metastasis	Right knee	HD-MTX, Adriamycin and cisplatin	5 g /m ² (6 g)

MTX was given i.v. over a period of 6 hours. Infusions were started in the afternoon and the routine blood sampling was done initially every 12 hours and later approximately every 24 hours. After about 5 days, sampling was done less frequently. Blood was collected in heparinized tubes and the plasma separated by centrifugation. The red cell pellet was washed three times with isotonic saline and then a hemolysate was prepared by adding three volumes of deionized water. Serum creatinine concentration was determined before and a couple of days after the administration of the drug to ensure its proper excretion.¹⁰ Non-specific binding of MTX at pH 7.2 by the preinfusion plasmas of both the patients was determined by a procedure described in detail in a previous communication¹² with the modification that no mercaptoethanol was used and instead of using cold MTX, increasing amounts of (3H) MTX (0.2 ng — 1.2 ng) were used in various reactions. A typical reaction mixture in a total volume of 0.5 ml in 0.06 M citrate, pH 7.2, contained NADPH 48 uM, (³H)MTX (0.2 ng — 1.2 ng) and 50 ul plasma. After 30 minutes incubation, 0.4 ml of 1% Norit A neutral charcoal in 0.5% Dextran (molecular weight 10,000) was added to each reaction. After centrifugation, radioactivity in 0.5 ml of supernatant solution which contained bound (3 H) MTX was counted in an LS—3801 spectrometer (Beckman Instruments Inc., Palo Alto, Calif.).

METHODS

Plasma MTX concentration was carefully monitored using a very sensitive radioassay¹¹ to assess the duration for which leucovorin therapy must be carried out to avoid toxicity from excessive retention of MTX. Total MTX was measured in washed erythrocytes after the cells had been hemolysed in three volumes of deionized water (vide supra) and the proteins precipitated by placing the hemolysate in a boiling water bath for 5 minutes. This extraction procedure has been described elsewhere.⁹ Protein-bound MTX in erythrocytes was determined by dialyzing the hemolysates overnight at 4°C against 0.15 M NaCl before extracting it from the samples by boiling.

RESULTS

Profiles of MTX clearance from plasma and erythrocytes of Patient # 1 (G.M) and Patient # 2 (S.H.) after a six hour MTX infusion are shown in Figures 1 and 2.

PLASMA & ERYTHROCYTE MTX

Profile of a Patient (G.M.) receiving 2 courses of HD-MTX therapy.

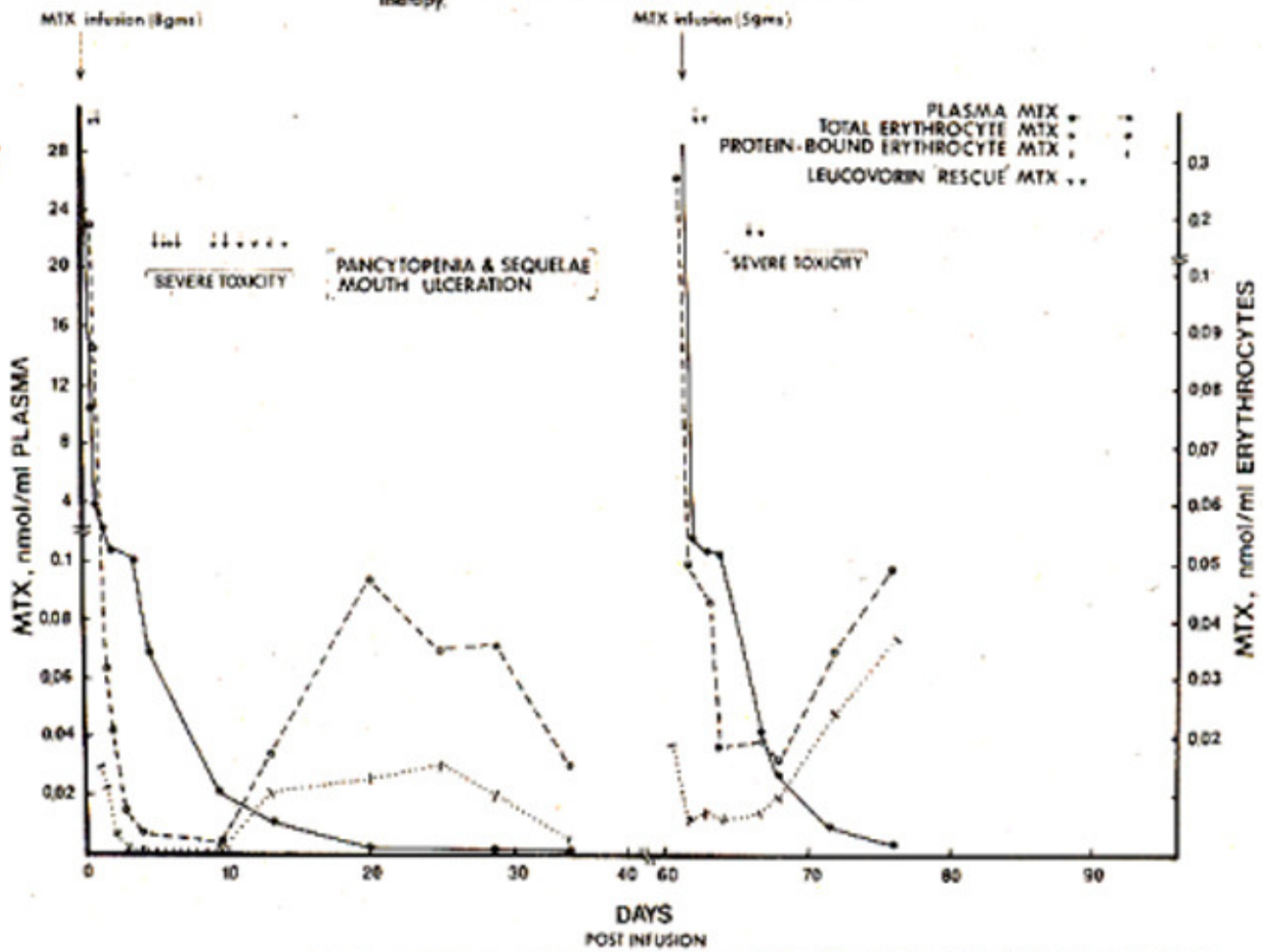


Figure 1. Plasma and erythrocyte MTX profile of a patient (G.M.) receiving 2 courses of HD-MTX therapy. During the first course, 8 gms of MTX was infused over a period of 6 hours. Second course of MTX therapy was given approximately 2 months after the first course and during this course 5g of the drug was infused over a period of 6 hours. Leucovorin 'rescue' was started exactly 20 hours after the MTX dose and involved 15mg tablets of leucovorin taken every 6 hours.

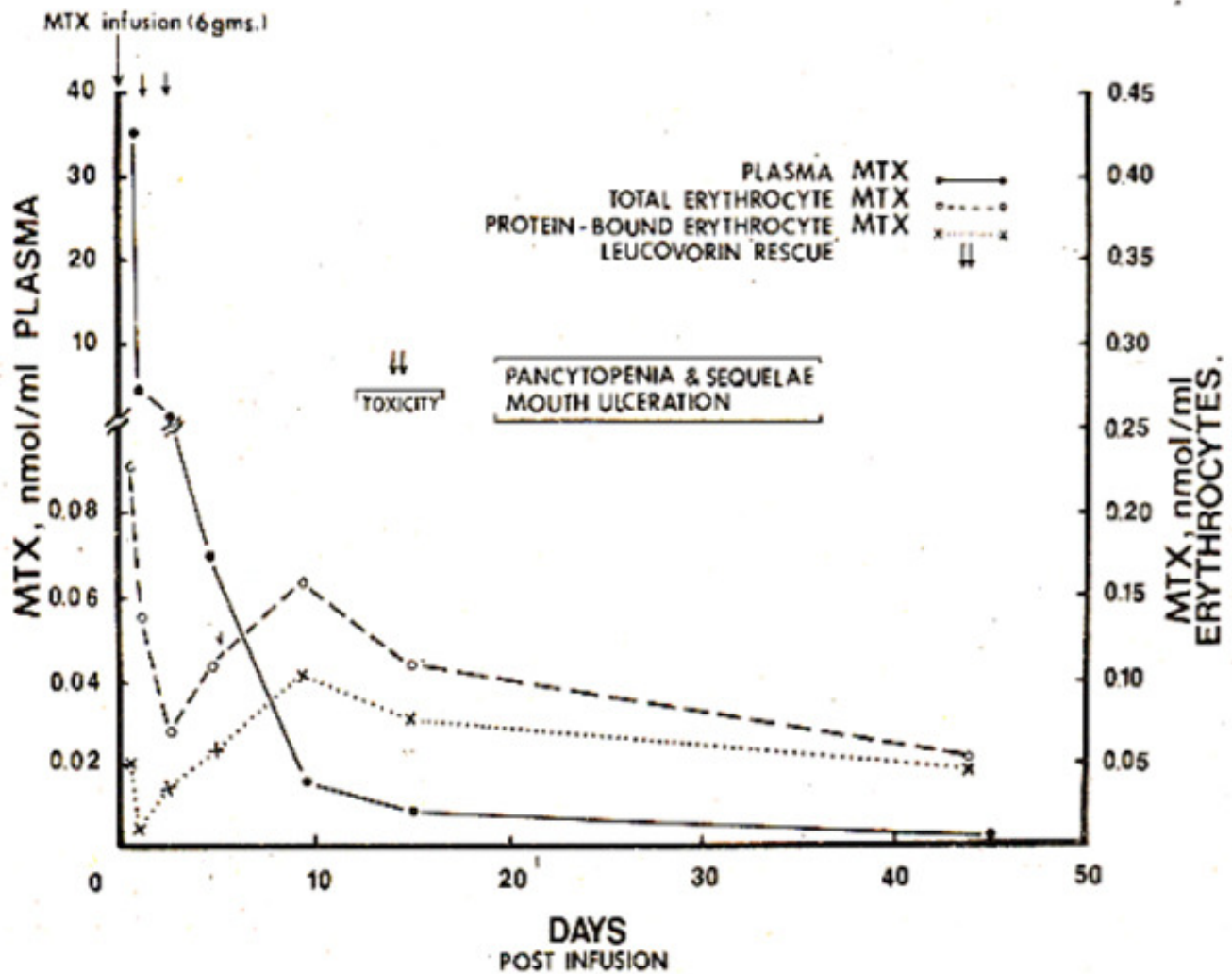


Figure 2. Plasma and erythrocyte MTX profile of a patient (S.H.) receiving an infusion of 6g of MTX. Leucovorin 'rescue' was started 20 hours after the drug and comprised 15mg tablets of leucovorin taken every 6 hours.

The elimination of the drug from plasma in both the cases appears to be triphasic. Such a profile for MTX-plasma decay has been reported by others as well^{13,14}. However, elimination of the drug during the first 36 hours turns out to be biphasic (Figure 3)

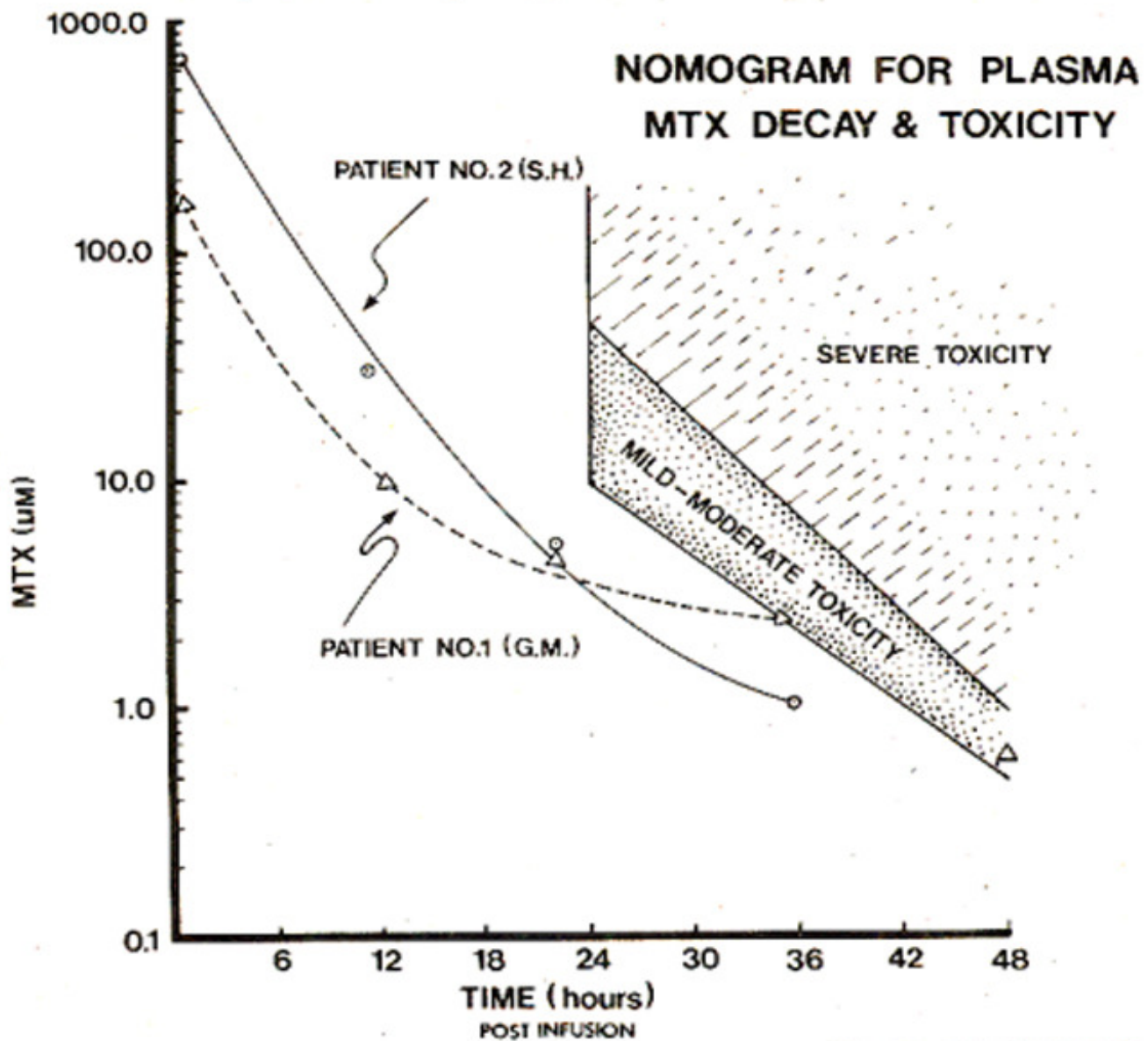


Figure 3. Decay of plasma MTX during the first 36 hours after a 6 hour infusion of the drug. Patient #1 (G.M.) received 8g MTX while Patient #2 was given 6g MTX. In the right hand corner, there is a nomogram for plasma MTX decay and toxicity. [For nomogram, see references 5 and 6].

with the initial phase of elimination considerably more rapid than the second phase. Using Figure 3 which shows the decay of plasma MTX after a 6 hour infusion, initial phase elimination half-lives ($T_{1/2\alpha}$) for patient # 1 (G.M.) and Patient 2 (S.W) were calculated to be approximately 225 hours and 325 hours, respectively, whereas the values for second phase elimination half lives ($T_{1/2\beta}$) in these two

patients were found to be around 5 hours and 6 hours. This indicates that the clearance of the drug from the plasma of patient # 2 (S.H.) is relatively slower than from the plasma of Patient #1 (G.M.). Figures 1 and 2 also show the changes occurring with time, in the levels of total and protein-bound MIX in erythrocytes. It is evident from the two profiles that in Patient # 1 (Figure 1) after day 9, and in Patient # 2 (Figure 2) after day 4 the levels of erythrocyte MTX increased while plasma MIX levels are rapidly decreased. In other words, there appears to be a reciprocal relationship between plasma MIX and erythrocyte MIX during this period of time. Moreover, most of the methotrexate associated with erythrocytes is protein bound probably to dihydrofolate reductase as it could not be removed by dialysis of the hemolysate. The elimination of the drug from erythrocytes was much slower compared with its clearance from plasma. Despite leucovorin therapy, both of our patients exhibited symptoms of severe toxicity which include pancytopenia and sequelae and mouth ulceration. It was surprising to note that the toxicity was less severe in Patient # 2 (S.H.) with relatively poor MIX clearance from plasma. In order to find out the probable cause of slower plasma clearance of the drug in Patient # 2, the preinfusion plasma samples of both the patients were checked for their ability to bind MIX nonspecifically. As shown in Figure 4,

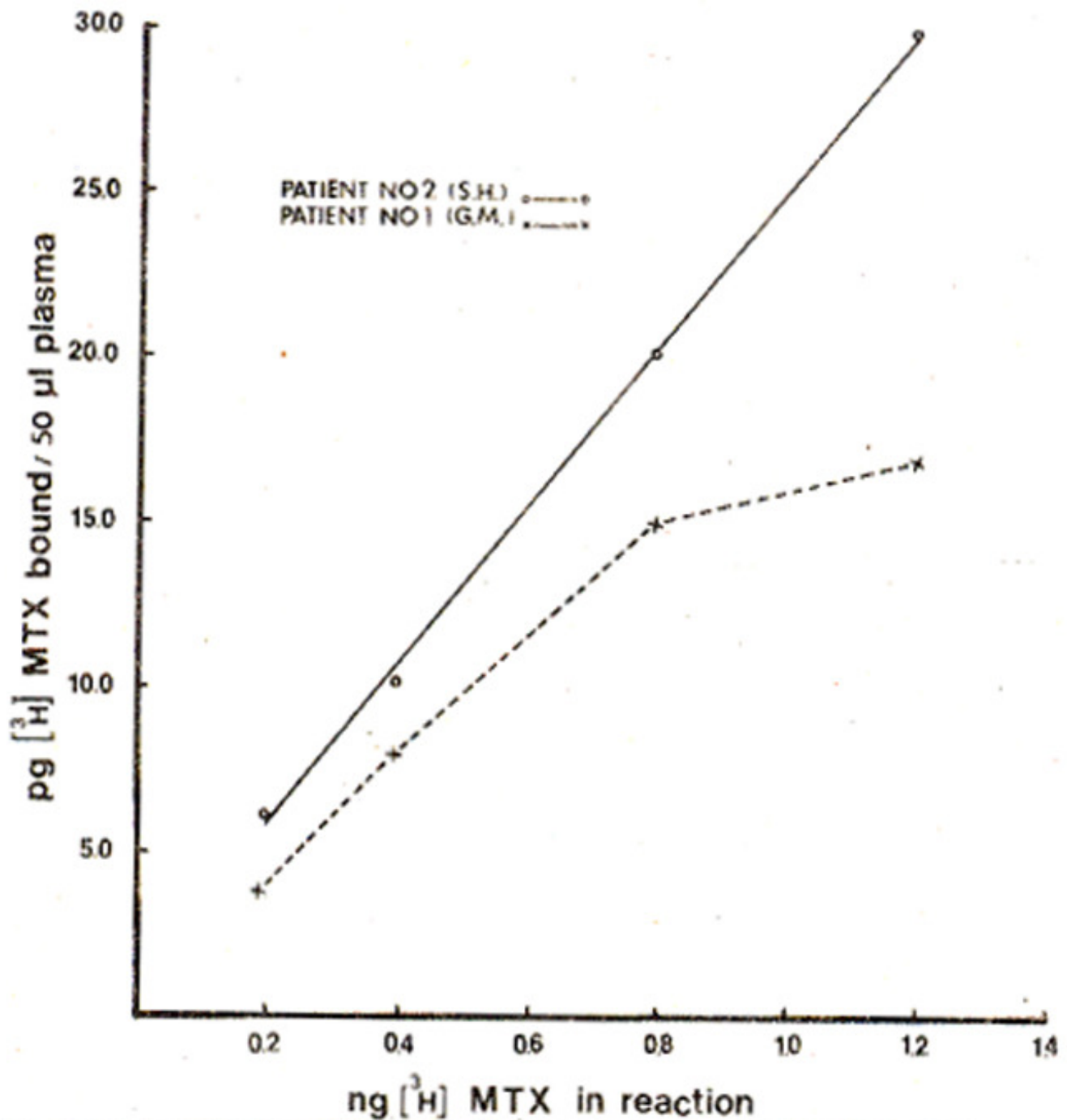


Figure 4. Nonspecific binding of MTX by preinfusion plasma samples of two patients. Details of the reaction are in Materials and Methods section.

the plasma from Patient #2 bound more MIX nonspecifically as compared to the plasma from Patient # 1. Therefore, nonspecific binding of the drug to plasma proteins may also have been one of the causes of delayed clearance of plasma MTX. To assess how avidly MTX was bound to these plasma proteins in these two patients, the plasma samples obtained 24 hours post HD-MTX infusion which were incidentally having nearly the same MTX concentrations (3.96 uM and 4.4 uM, respectively), were

extensively dialyzed at 4°C against 0.15 NaCl. It was interesting to note that Patient # 1 (G.M.) with better MTX clearance from plasma contained more nondialyzable MTX (0.22 μM) compared with Patient # 2 (0.04 μM) suggesting that the concentration of nondialyzable protein-drug complexes or the fraction, of the drug tightly bound to plasma proteins has little correlation with plasma clearance of the drug.

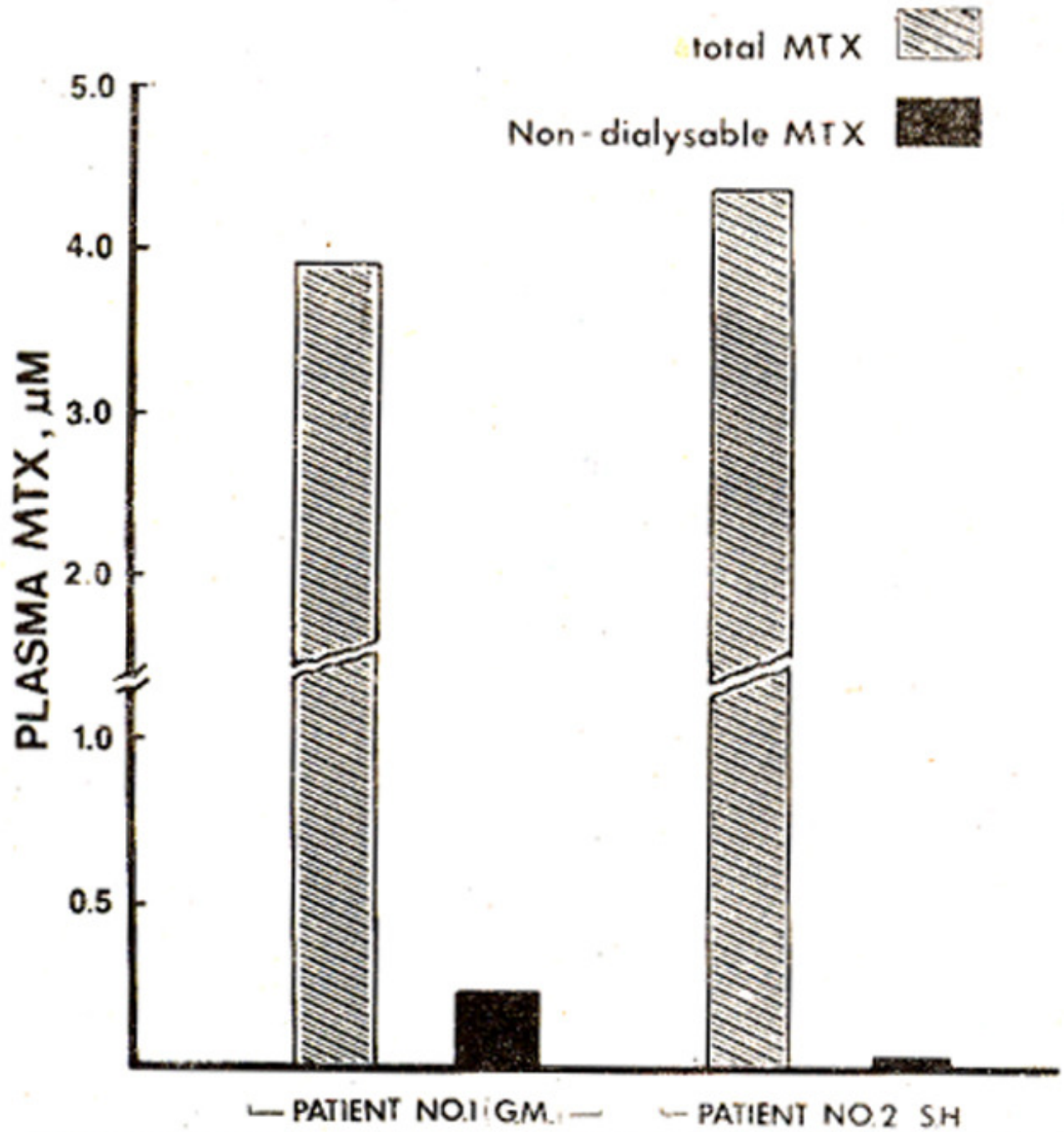


Figure 5. Plasma MTX (dialyzable and nondialyzable) levels in two patients 24 hours post HD-MTX infusion (see the text for details).

DISCUSSION

HD-MTX therapy with leucovorin rescue has been in use for more than a decade. The ultimate value of this treatment in osteogenic sarcoma is not fully established. However, therapeutic benefits in terms of delay in recurrence following surgery or a partial response of metastatic disease has been documented in a situation where there are few other alternatives. Such a study is probably the first of its kind carried out in Pakistan. The two patients responded reasonably well. There has been no relapse of the disease in one of them who was treated nearly 8 months ago. With close monitoring and adequate supportive therapy, the regimen used was reasonably safe. Since the principal mode of elimination of MTX is via the kidney, it is essential to ensure the integrity of renal function before scheduled administration of the drug. Serum creatinine concentration has often been used to predict impaired excretion of MTX.¹⁰ Therefore, we used this parameter to ensure proper renal function. In both of our patients, the values of serum creatinine remained well within normal limits during the course of the treatment. Plasma MTX decay curves after infusions of the drug appear to be biphasic during the first 36 hours (Figure 3). The half life values of MTX for two patients during the “initial” phase were 2.25 hours and 3.25 hours respectively which correlate well with the value of 2 — 3.5 hours as reported by others^{15,16}. However, the “second” phase elimination half-life values (5 and 6 hours) obtained in our patients were somewhat lower than the values (7 — 10 hours) reported by some other investigators^{15,16}. The reappearance of the drug in circulating erythrocytes, 4 — 9 days after infusion, indicates that it is incorporated into the proliferating bone marrow erythroblasts. The time it takes to appear again in the circulating erythrocytes represents the time required for intramedullary maturation of erythroid precursor cells into mature erythrocytes. Our results are consistent with the previous studies on this subject^{8,9} and further confirm that erythrocytes do function as a slow-changing compartment for MTX because in our study too, the terminal half-life of the drug was 2-5 weeks. Our results also indicate that the erythrocyte MIX phenomenon is commonly observed even in patients receiving a single high-dose infusion of the drug. It was surprising to note that both of our patients exhibited symptoms of severe toxicity although according to the nomogram (Figure 3) their plasma MTX values were well below the levels generally considered to be related to severe toxicity^{5,6}. We did not find any direct correlation between plasma levels of MTX and the clinical toxicity. In our study, clinical toxicity was more pronounced in a patient with better clearance of the drug. Recently there have been a couple of reports suggesting that decreased production of 7—OH—MIX in the sera of patients receiving HD—MTX therapy seems to be correlated with severe clinical toxicity.¹⁷ 7—OH—MIX is a derivative of MTX produced in the body following administration of MIX. It is a 200-fold less potent inhibitor of dihydrofolate reductase than MTX. Significant production of 7—OH—MIX following LID—MIX therapy may contribute to the rescue of tissue from MIX intoxication. Studies are being carried out to further explore this phenomenon. The results of our study lend support to the notion that clinical toxicity may not be predictable by monitoring MIX alone, and other parameters, such as decreased generation of 7—OH—MIX may also have to be taken into account when predicting MIX toxicity.

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