In Stage 1 melanoma of the limbs, delayed dissection of lymph nodes (i.e., performed at the time of appearance of regional metastases) is as effective in the control of the disease as immediate dissection. Since the proportion of positive nodes ranges from 20 to 25 per cent, the "wait-and-see" policy avoids unnecessary postoperative complications in three fourths of the patients, for whom the "prophylactic" dissection would result in negative histologic findings in regional nodes. Delayed dissection is advisable as long as the patient can be kept under strict clinical control.

We are indebted to the surgical and pathological staffs of the institutes listed for their co-operation, particularly to Dr. A. Levene, London, chairman of the pathologists' panel for the care taken in the diagnostic confirmation of cases and for his co-ordination of the panel's work, and to Dr. A. Breslow, Washington, D.C., and Dr. E. P. van der Esch, Amsterdam, Netherlands, for the reclassification of cases according to maximum thickness and levels of invasion.

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USE OF PLASMA PHARMACOKINETICS TO PREDICT AND PREVENT METHOTREXATE TOXICITY

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Abstract To correlate the pharmacokinetics and toxicity of methotrexate, we measured the drug's clearance from plasma after 395 high-dose, six-hour infusions given to 78 patients. After 375 infusions, 48-hour methotrexate levels fell within 2 standard deviations of the mean for nontoxic infusions, and myelosuppression did not occur. Methotrexate concentrations exceeded the range for nontoxic patients (mean ±2 standard deviations) after 20 infusions. Serious myelosuppression occurred after six of these 20 infusions, including five of 12 infusions associated with 48-hour drug concentrations above 9×10^{-7} M. In seven patients with 48-hour concentrations above 9×10^{-7} M, the absence of toxicity could be attributed to subsequent rapid clearance of the drug; four of these patients also received large doses of supplemental leucovorin (50 to 100 mg per square meter every six hours). Determination of methotrexate concentration in plasma thus identified patients at high risk of toxicity, a group that may benefit from supplemental leucovorin rescue. (N Engl J Med 297:630-634, 1977)

METHOTREXATE, an effective agent in treatment of leukemia, choriocarcinoma and other tumors, is one of the most versatile antineoplastic agents because its toxicity in high-dose regimens can be prevented by the reduced folate, leucovorin (5-formyltetrahydrofolate).[1] High-dose methotrexate regimens (50 mg per kilogram or more) with leucovorin rescue have produced responses in metastatic osteogenic sarcoma[2] and other tumors[3] and have lengthened the relapse-free interval when used as an adjuvant after surgical excision of nonmetastatic osteogenic sarcoma.[4]

High-dose methotrexate therapy has not been without serious complications. Although the majority of such infusions cause only minor side effects, severe and prolonged myelosuppression and mucositis have developed in approximately 10 per cent of patients, and fatalities directly attributable to drug toxicity have occurred in 29 of 498 patients (6 per cent) treated with high-dose methotrexate, according to a recent survey of cancer treatment centers in the United States.[5] Experimental studies have implicated methotrexate-induced renal dysfunction and delayed drug clearance as a probable mechanism of toxicity,[6-8] and initial attempts to monitor drug levels in patients receiving high-dose therapy have demonstrated delayed drug clearance in toxic patients.[6,7] These considerations suggested that routine monitoring of methotrexate levels in plasma might permit early detection of patients at high risk of development of toxicity. The following study substantiates the value of monitoring plasma methotrexate 48 hours after drug administration.

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Materials and Methods

From November, 1974, to January, 1977, 78 patients at the National Cancer Institute received 395 infusions of high-dose methotrexate for treatment of metastatic tumors (38 patients) or as adjuvant therapy for osteogenic sarcoma (40 patients). Their median age was 25 years, with a range of 10 to 70 years. Treatment protocols required that all patients receiving high-dose methotrexate have a normal blood urea nitrogen (less than 25 mg per 100 ml) and normal serum creatinine (less than 1.2 mg per 100 ml) before each course of therapy. In addition, all patients had a white-cell count greater than 4000 cells per cubic millimeter and platelet count greater than 150,000 per cubic millimeter before treatment, except for Patient 3 (Table 1), who had a platelet count of 16,000 cells per cubic millimeter before treatment.

Myelosuppression due to methotrexate was defined as a fall of the white-cell count to 2000 cells per cubic millimeter or less, or a fall in platelet count to 75,000 cells per cubic millimeter or lower. Drug-related renal toxicity was defined as a 50 per cent or greater rise in serum creatinine, the peak value exceeding the normal limits of 1.2 mg per 100 ml.

Methotrexate Infusion

The regimen previously reported by Jaffe et al.[4] was used in this study. Methotrexate was supplied by Lederle Laboratories, Pearl River, New York, or Ben Venue Laboratories, Bedford, Ohio, and was administered in 500 to 1000 ml of 5 per cent dextrose in water containing 25 meq of sodium bicarbonate.

Methotrexate in doses of 50, 100, 150, 200, or 250 mg per kilogram was infused intravenously over a six-hour period. All patients received intravenous hydration with 3 liters of fluid per square meter per 24 hours and 80 meq of bicarbonate per square meter per 24 hours. Hydration was begun 12 hours before and was continued for 36 hours after the start of the infusion. Urinary pH was tested before and periodically during the 24 hours after the start of the infusion. Methotrexate was not begun until the urinary pH was greater than or equal to 7.0, and additional intravenous bicarbonate was recommended if the urinary pH fell below 7.0 during infusion.

Leucovorin Rescue

Leucovorin, 15 mg per square meter, was given intravenously two hours after the completion of methotrexate infusion and was repeated every six hours thereafter for a total of eight doses. At 48 hours from the start of methotrexate infusion plasma samples were obtained for methotrexate determination. Plasma methotrexate concentrations were determined either by the dihydrofolate reductase inhibition method using enzyme from either *Lactobacillus casei* (New England Enzyme, Boston, Massachusetts) or from methotrexate-resistant murine L1210 cells,[9] or by the competitive protein-binding assay.[10] Both assays are highly specific for tight-binding inhibitors of dihydrofolate reductase and yield equivalent results when tested against known standards or the same patient sample. Pharmacokinetc data from the first 14 nontoxic infusions were used to initially define a normal range (mean ±2 S.D.) for 48-hour plasma methotrexate concentration. A normal 48-hour plasma methotrexate range was later established for each dose level administered on the basis of data from the first 189 nontoxic infusions. Patients whose 48-hour level fell within this normal range were discharged from the hospital, whereas those with levels more than 2 S.D. above the mean received leucovorin for an additional 48 hours. During the initial 18 months of this study, leucovorin dosage was 12 to 30 mg per square meter every six hours when additional rescue was given. In the final six months of the study, higher doses of supplemental leucovorin, 50 to 100 mg per square meter every six hours, were used.

Results

Initial pharmacokinetic studies were performed in 14 patients who received 50 to 250 mg per kilogram of methotrexate without subsequent toxicity. Peak drug levels were 0.1 mM to 1.0 mM during the infusion period and declined thereafter in a biexponential curve with half-lives of two and 10.4 hours (Fig. 1). Plasma methotrexate concentrations were 3×10^{-7} M or less in all patients 48 hours after the infusion was begun. We suspected that toxic patients would display pro-

Table 1. Toxic Episodes Due to High-Dose Methotrexate (MTX).

Patient No.	Dose	48-Hr Plasma MTX	Serum Creatinine		Supplemental Leucovorin	Toxicity*			Recovery†
			Before MTX	After MTX		White-cell count	Platelet count	Other	
	mg/kg	*μM*	*mg/100 ml*		*mg/m²/6 hr*				
Myelosuppression:									
1	50	1.4	0.8	1.3 (96 hr)		2,200 (day 6)	54,000 (day 10)	Urinary-tract infection	On day 12
2	50	21.0	1.2	2.6 (24 hr)	6 (day 2–22)	1,700 (day 7)	23,000 (day 13)	Severe mucositis	On day 17
3	100	6.9	0.9	1.5 (24 hr)	30 (day 2–13)	500 (day 6)	500 (day 8)	Septicemia	Patient died on day 13
4	100	1.4	0.8	0.8 (48 hr)	15 (day 5–11)	1,600 (day 7)	60,000 (day 7)	Rash, severe mucositis	On day 14
5	100	0.25 (72 hr)	0.7	1.0 (24 hr)	20 (day 2–17)	2,300 (day 6)	15,000 (day 11)	—	On day 23
6	150	0.96	0.6	0.5 (48 hr)	15 (day 2–5)	1,700 (day 4)	No fall	—	On day 5
Renal toxicity with moderate leukopenia:									
7	50	0.96	1.1	2.7 (48 hr)	75 (day 2–21)	3,200 (day 7)	163,000 (day 10)	—	—

*White-cell count ≤2000 cells/mm³ or platelet count ≤75,000 cells/mm³.

†White-cell count >2000 cells/mm³, platelet count >75,000 cells/mm³, & disappearance of mucositis.

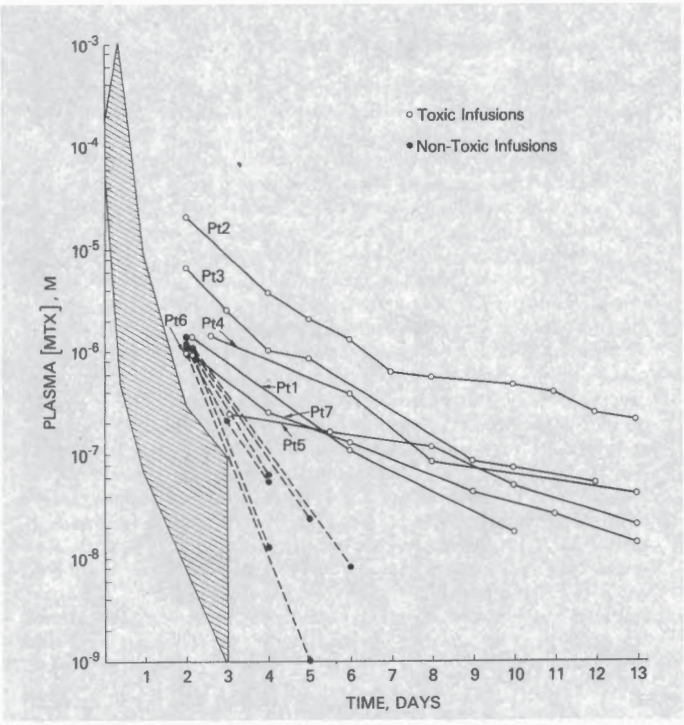

Figure 1. Plasma Methotrexate (MTX) Disappearance Curves in Patients Receiving Six-Hour Infusions, 50 to 250 Mg per Kilogram.

The disappearance profile for 14 patients monitored serially over 72 hours who had no toxicity is defined by the striped area. Patients in whom evidence of myelosuppression developed (Table 1) had plasma levels as shown by open circles. Six nontoxic patients with 48-hour levels above 0.9 μM had the plasma levels as shown by the solid circles.

longed retention of methotrexate; therefore, plasma samples were routinely monitored 48 hours after the start of therapy in an effort to detect patients at high risk of toxicity.

In 375 of 395 infusions, plasma methotrexate concentrations at 48 hours fell within the normal range, as defined by the geometric mean ±2 S.D. for the nontoxic patients in this series (Fig. 2). None of these infusions associated with normal 48-hour levels resulted in myelosuppression, mucositis, or other toxicity. After seven infusions, the 48-hour plasma level exceeded the normal range but was less than 9×10^{-7} M; in each case, the patient received supplemental leucovorin, 15 mg per square meter every six hours for two days, and no patient manifested toxicity.

Forty-eight-hour levels of methotrexate greater than 9×10^{-7} M were associated with a high frequency of toxicity. In five of 12 patients having 48-hour values above 9×10^{-7} M severe myelosuppression developed (Table 1). An additional toxic episode occurred in a patient with high levels that were first detected at 72 hours after infusion (Patient 5, Table 1).

Six patients with 48-hour levels greater than 9×10^{-7} M did not show toxicity. Two factors distinguished the toxic patients from patients having similarly elevated drug levels at 48 hours but no subsequent toxicity. The toxic patients demonstrated markedly delayed drug excretion when additional methotrexate levels were determined at 72 and 96 hours (Fig. 1); in contrast, the nontoxic patients all had rapid clearance of the drug, as verified by serial determinations.

Secondly, dosage of leucovorin may have influenced toxicity in that the severely toxic patients (Patients 1-6, Table 1) all received supplemental leucovorin in low doses (12 to 30 mg per square meter every six hours) whereas four of the nontoxic patients received larger doses of supplemental leucovorin (50 to 100 mg per square meter every six hours). Further evidence for the effectiveness of large doses of leucovorin was provided by the course of Patient 7 (Table 1). In this patient renal failure associated with markedly delayed methotrexate disappearance from plasma developed; he received leucovorin, 75 mg per square meter every six hours for 19 days, and had moderate

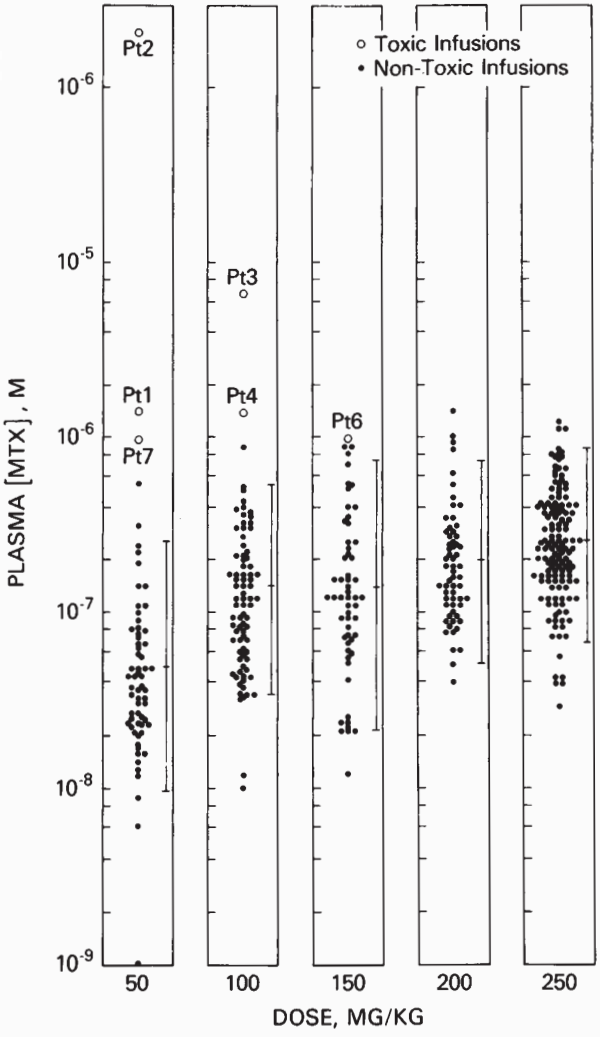

Figure 2. Relation of Plasma Methotrexate (MTX) Level at 48 Hours to Toxicity.

The geometric mean values of nontoxic infusions are shown at each dose level, with brackets defining 2 S.D.

granulocytopenia as the sole manifestation of drug toxicity.

Myelosuppression in the toxic patients continued for five to 23 days and, in two (Patients 2 and 4, Table 1), was accompanied by severe oral mucositis. Other toxic sequelae included a diffuse erythematous rash in Patient 4, a urinary-tract infection in Patient 1, and gross hematuria followed by a fatal episode of septicemia in Patient 3.

Value of Serum Creatinine in Predicting Methotrexate Toxicity

Jacobs et al.[7] have shown in monkeys that, as a consequence of its limited solubility at acid pH, methotrexate may precipitate in acid urine, causing renal tubular obstruction and delayed drug excretion. Pitman et al.[8] have proposed that serum creatinine increase of 50 per cent might be used to detect patients at high risk of development of toxicity after high-dose methotrexate.

In the present study all patients had normal serum creatinine values before treatment. Post-infusion creatinine values, monitored at 24 or 48 hours, did not change or increased less than 50 per cent in 383 of 395 infusions. Despite the unchanged creatinine, three of these infusions were associated with myelosuppression. An increase of more than 50 per cent in serum creatinine occurred after 12 infusions; in four cases the peak creatinine value exceeded normal limits, and each of these infusions was associated with myelosuppression. In the other eight patients, despite the 50 per cent increase after treatment, the absolute creatinine value did not exceed normal (1.2 mg per 100 ml), and none of these patients became toxic. Thus, if one relied on the criterion of an increase in creatinine of more than 50 per cent to an abnormal peak value, it would have been possible to predict toxicity in only four of seven toxic patients.

Discussion

The use of high-dose methotrexate infusions,[2-6] although of great interest in cancer therapy, poses the potential of life-threatening toxicity for the patient.

The present study has demonstrated that monitoring of plasma methotrexate levels 48 hours after infusion can identify patients at high risk of toxicity. Six patients who experienced myelosuppression had levels at 48 hours higher than 9×10^{-7} M, and had markedly delayed excretion of drug thereafter. A seventh toxic patient had an elevated level at 72 hours and delayed drug disappearance. In contrast, no patient having a level less than 9×10^{-7} M at 48 hours experienced subsequent toxicity. Six patients with 48-hour levels higher than 9×10^{-7} M did not show toxicity. The avoidance of toxicity by these six patients could be explained by either the subsequent rapid fall off in drug levels in all six patients or the high-dose leucovorin that four of the six received.

Other studies have suggested that an increase in serum creatinine may be useful as an early sign of impending toxicity.[8] However, in the present series, a rise in creatinine to abnormal levels was observed in only four of seven toxic patients. This finding suggests that if precipitation of methotrexate in renal tubules underlies toxicity, serum creatinine measurements may not be sensitive enough to detect this functional impairment in all cases. Alternatively, other unidentified factors, such as individual differences in renal tubular function or drug metabolism, might account for the delayed drug excretion and toxicity.

The management of patients with elevated methotrexate levels and at high risk of toxicity has not been resolved. The present study provides evidence that large doses of leucovorin may prevent toxicity. In none of five patients who received more than 50 mg per square meter of leucovorin beginning at 48 hours did severe mucositis or myelosuppression develop. In further support of this possibility, one patient (Patient 7, Table 1), who had evidence of renal failure and markedly delayed drug excretion, experienced only mild leukopenia when supported with leucovorin, 75 mg per square meter every six hours for 21 days.

In experimental systems, the concentration of leucovorin required to prevent toxicity increases in direct proportion to the concentration of methotrexate,[11] but the specific levels required to counteract specific methotrexate concentrations are not known. Because methotrexate concentrations above 1×10^{-8} M inhibit DNA synthesis in bone marrow and intestinal epithelium,[12] it is reasonable to continue leucovorin rescue in toxic patients until plasma methotrexate falls below this level. Measures designed to accelerate removal of methotrexate, such as hemodialysis,[13] charcoal filtration[13] or enzymatic cleavage,[14] might have considerable value in patients with abnormal renal function, but have not been carefully evaluated in man as yet.

The foregoing results demonstrate the value of monitoring plasma methotrexate levels during high-dose chemotherapy. The specific correlates of toxicity, including critical plasma levels and duration of exposure, will probably differ from regimens employing other schedules of methotrexate administration. Nonetheless, it is clear that determinations of drug levels will be of value for identifying patients at high risk after methotrexate therapy.

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AUTOIMMUNE VITILIGO

Detection of Antibodies to Melanin-Producing Cells

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Abstract Vitiligo, a disorder characterized by the destruction of melanocytes, is often associated with diseases in which there are increased frequencies of autoantibodies. For this reason we investigated two patients with vitiligo, alopecia universalis, mucocutaneous candidiasis, and multiple endocrine insufficiencies for antibodies to melanin-producing cells. Using direct immunofluorescence of normal and vitiliginous skin from both patients and indirect immunofluorescence with both patients' serum, we could not detect these antibodies. However, an immunofluorescent complement-fixation test demonstrated a circulating antibody that bound to melanocytes in human skin, nevus cells and melanoma cells. Specificity of cellular fluorescence for nevus and melanoma cells was shown on serial sections stained with hematoxylin and eosin and was inferred for melanocytes from their distribution in human skin and their presence when the normal but not vitiliginous skin of both patients was used as substrate. This antibody was characterized as an IgG that activated complement via the classical pathway. (N Engl J Med 297:634-637, 1977)

VITILIGO, a disease characterized by the loss of melanin pigment that follows the destruction of melanocytes, has been associated with hyperthyroidism, hypothyroidism, hypoparathyroidism, pernicious anemia, diabetes mellitus, mucocutaneous candidiasis and alopecia areata. In addition to numerous case reports relating its occurrence with one or several of these disorders,[1-6] larger studies have confirmed these associations.[7-11] While the clinical associations of vitiligo have led some to consider it a cutaneous marker for internal disease,[12] the presence of thyroid, adrenal and gastric parietal-cell antibodies in many patients with both vitiligo and endocrine disease has suggested a common, perhaps autoimmune, origin.[1,3,6,11] The autoimmune theory of vitiligo is further strengthened by the increased prevalence of organ-specific autoantibodies in several,[7,13-15] but not all,[16] large series of patients with vitiligo.

To date, an antimelanocyte antibody in the serum of patients with vitiligo has not been found.[17] In the two patients with vitiligo, alopecia universalis, mucocutaneous candidiasis and multiple endocrine insufficiencies described below, we demonstrated a circulating antibody to melanocytes, nevus cells and melanoma cells. The antibody is identified by its complement-fixing ability and has been characterized as an IgG.

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CLINICAL SUMMARIES

CASE 1. A 34-year-old woman has had widespread candidiasis and total alopecia since the age of six. Hypoparathyroidism had been diagnosed at nine years of age, and the evaluation of an unsteady gait had led to the recognition of pernicious anemia at the age of 13. Her first menstrual period had occurred at the age of 15, but subsequent periods were infrequent, leading to the diagnosis of primary ovarian failure. Vitiligo began at the age of 28 and has been progressive; the most striking areas of involvement have been the face and extremities. Hypoadrenalism was diagnosed during her initial admission to the National Institutes of Health at the age of 34.

CASE 2. An 18-year-old woman has had candidiasis of her nails, total alopecia and vitiligo since three years of age, hypoparathyroidism since six and hypoadrenalism since 10. Primary ovarian failure was diagnosed at the age of 16; approximately 50 per cent of her skin was vitiliginous at that time.

Both patients have had extensive immunologic evaluations disclosing impaired cellular immunity to *Candida albicans* in vivo and in vitro. Serum autoantibody studies showed Case 1 to have thyroid complement-fixing and adrenal antibodies but no thyroid-agglutinating or gastric parietal-cell antibodies. Case 2 had adrenal, gastric parietal-cell and ovarian antibodies.

METHODS

Immunofluorescence. Immunofluorescent staining was performed with previously described procedures and reagents.[18] We examined biopsies of normal, vitiliginous, and border skin from both patients by direct immunofluorescence for in vivo bound IgG, IgA, IgM, IgE or C3. Serum samples from both patients were studied by conventional indirect immunofluorescence for the presence of circulating antibodies (IgG, IgA, IgM or IgE) and by an immunofluorescent complement-fixation test for circulating complement-binding factors capable of reacting with melanin-producing cells in normal or neoplastic tissue. The complement-fixation test is a multistep procedure involving sequential incubation of frozen sections of sub-