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Diurnal Variation of Methotrexate Pharmacokinetics in Adults With Osteosarcoma

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Abstract: The daytime and nighttime dispositions of high dose (12 g/m^2) methotrexate were examined in 8 osteosarcoma patients. The plasma and renal clearances, urine pH values and area under the curves were compared. The plasma clearance of methotrexate tended to fall after it was administered at (from 1.74±0.06 ml/min/kg to 0.47±0.01 ml/min/kg, $p<0.05$). Renal clearance (from 2.48±0.54 ml/min/kg to 1.18±0.3 ml/min/kg, $p<0.05$) and urine pH (from 7.14±0.24 to 6.16±0.26, $p<0.05$) decreased significantly, whereas no significant changes were observed in creatinine clearances. The area under the plotted curves increased from 220.76±28.32 to 759.71±99.56 mg/ml/min ($p<0.05$) after night-time exposure. These results suggest that the fall in urine pH at night results in decreased renal clearance of methotrexate.

Key Words: Methotrexate, diurnal variation, osteosarcoma

Introduction

Methotrexate and other folic acid antagonists have been extensively used for a variety of solid tumors and haematologic malignancies (1, 2). High dose methotrexate followed by leucoverin has become an important mode of therapy in childhood leukemias (3). Recent studies have suggested the importance of dosage and schedule on the therapeutic efficacy of this drug. Regimens vary from conventional low doses to extremely high doses, which carry the risk of toxicity depending upon the kind of malignancy being treated. The risk of complications and difficulties in maintaining complicated protocols necessitate regular monitoring of the drug. The most common side effects of methotrexate are renal and hepatic disfunction, nausea and vomiting, depression of the bone marrow, stomatitis, gastrointestinal mucositis, transient hepatitis-like syndrome and interstitial pneumonitis (4). The severity of toxicity depends upon the concentration of the drug and time of the therapy (5). Therefore, recommendations for dosage based on concentration-time curves are extensively applied in high-dose methotrexate treatment. Several studies have shown that adjustments in dosage and procedure have dramatically increased the therapeutic efficacy of methotrexate.

In a recent study, Rivard et al. (6) described better results in children with leukemia who received their maintenance theraph at night rather than in the morning. However, other authors found no difference in the pharmacokinetics of methotrexate administered during the day and at night (7).

The aim of this study was to investigate the possible alterations in methotrexate disposition after day-time and night-time administration in adult osteosarcoma patients.

Material and Methods

We compared the disposition phamacokinetics of high-dose methotrexate (Methotrexate Roger Bellon; Eczacıbaşı Rhone-Poulenc, France) administered in the morning (8.00 a.m.) and in the evening (8.00 pm) in 8 male osteosarcoma patients between the ages of 18 and 23. The patients were treated with intravenous (i.v.) methotrexate (12 g/m^2) given through a 6-hour infusion according to the protocol of the Mayo Clinic North Central Cancer Treatment Group (NCCTG). In order to prevent toxicity, the patients were given folinic acid (Calcium-Leucoverin; Orna, David Bull, Australia) (15 mg/6 hours/parenterally) 24 hours after the methotrexate. The leucoverin dosage was adjusted according to serum

methotrexate levels.

Blood was drawn through a heparinized needle just before and 3, 6, 24, 48 and 72 hours after the methotrexate infusion. Urine samples collected over a 24-hour period were stored the in dark bottlos to protect the specimens from light. Methotrexate concentrations in both the serum and urine were measured by Enzyme Multiplied Immune Technology (EMIT). The EMIT assay is a homogenous enzyme immunassay technique used for the analysis of specific compounds in biological fluids. The assay is based upon competition between the drug in the sample and a drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts oxidized nicotinamide dinucleotid (NAD) to NADH, resulting in an absorbance change that is measured by spectrophotometry. Endogenous serum G6P-DH does not interfere because the coenzyme functions only with the bacterial enzyme employed in the assay (8).

The area under the curves (AUC) was calculated by a PharmCalc. program with the use of the trapezoidal method (9).

The renal and plasma clearances of methotrexate were calculated using the following equations:

Renal clearance=Drug concentration in urine/$AUC_{(0\text{-}24)}$

Plasma clearance=Drug dose/$AUC_{(0\text{-}24)}$

The results are expressed as means±standard error (S.E.M).

Statistical analysis was performed by Student's (*t*) test.

Results

The plasma clearance of methotrexate was lower after nightime administration of the drug (0.47±0.01 ml/min/kg, $p<0.05$) than after daytime administration (1.74±0.06 ml/min/kg). Renal clearance (from 2.48±0.54 ml/min/kg to 1.18±0.3 ml/min/kg, $p<0.05$) and urine pH (from 7.14±0.24 to 6.16±0.26, $p<0.05$) decreased significantly after nighttime administration, whereas no significant changes were observed in creatinine clearances. The area under the curves increased from 220.76±28.32 mg/ml/min. to 759.71±99.56 mg/ml/min. ($p<0.05$) after nighttime exposure. None of the patients exhibited signs of serious toxicity.

Discussion

The diurnal variation in methotrexate pharmacokinetics has been investigated by several authors. In a clinical trial in children with leukemia, Ferrazzini et al. (10) showed that the clearance rate of methotrexate was slower at night and that systemic exposure to the drug was larger. Rivard et al. (6) also reported significantly improved results with nighttime administration of the drug in children with leukemia.

In the present study, we found a significant fall in the renal clearance of methotrexate after nighttime administration. In addition, the AUC was significantly greater than that for day-time exposure.

The elimination of methotrexate involves glomerular filtration, tubular secretion and reabsorption mechanisms. Tubular secretion occurs via the organic anion transpors system (11). Diurnal changes in methotrexate clearance are not attributed to variations in the glomerular filtration rate, but to a reduction in net tubular secretion. Methotrexate is a weak organic acid and its tubular reabsorption depends on changes in urinary pH (12). A fall in urinary pH from 6.9 to 5.7 decreases its solubility from 20 μmol to 2 μmol (13). At night, urinary pH is more acidic (14), and this causes more reabsorption of the less ionized drug, resulting in reduced renal clearance. During the day, urine pH tends to be more alkaline, which may cause ion trapping of the drug, resulting in reduced reabsorption and increased renal clearance. This is in agreement with the mechanism proposed by Waterhouse and Minor (15). However, these findings are in contrast with the results of Billis et al., who found no differences between the daytime and nighttime pharmacokinetics of methotrexate. The differences in these results are probably due to the fact that methotrexate was administered orally in the latter study and possible changes in the absorption of the drug may have masked the diurnal changes in methotrexate clearance.

In conclusion, our data suggests that diurnal changes in urinary pH cause alterations in the renal clearance of methotrexate. Further studies are necesarry to investigate the effect of these changes on the efficacy of

	Day	Night	p value
Plasma clearance (ml/min/kg)	1.74±0.06	0.47±0.01	p<0.05
Renal clearance (ml/min/kg)	2.48±0.54	1.18±0.3	p<0.05
Creatinine clearance (ml/min/kg)	1.01±0.03	1.00±0.03	N.S.
AUC (mg/ml/min)	220.76±28.32	759.71±99.56	p<0.05
Urine pH	7.14±0.24	6.16±0.26	p<0.05

Table 1. Comparison of parameters after day-time and night-time administration of methotrexate in adult osteosarcoma patients.

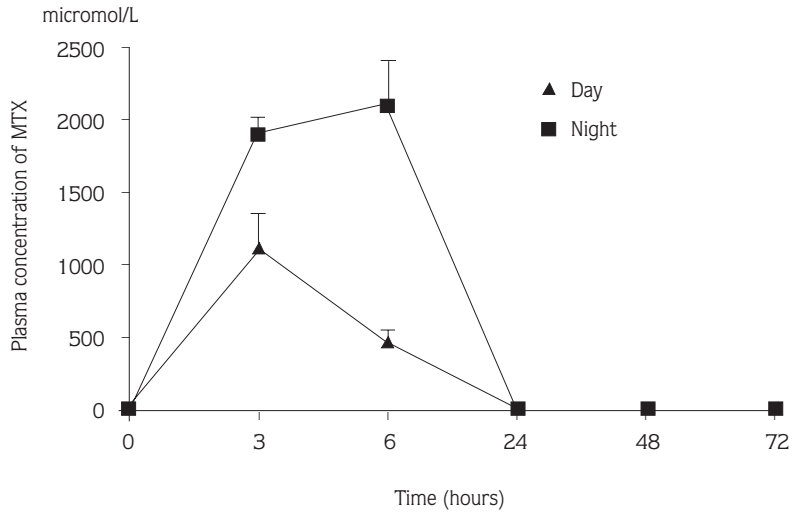

Figure 1. Plasma levels of methotrexate after day-time and night-time administration of the drug in adults with osteosarcoma.

the drug.

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