Proc EDTA–ERA (1985) Vol 22

NEPHROTOXICITY OF HIGH DOSE METHOTREXATE THERAPY – A LONG-TERM FOLLOW-UP STUDY IN FIVE JUVENILE PATIENTS WITH OSTEOSARCOMA

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Summary

Nephrotoxic side effects of high dose methotrexate (MTX) therapy were investigated in five patients aged eight to 22 years during eight to 18 (mean 14.6) cycles of treatment in nine months. Creatinine clearance decreased to less than 40ml/min/1.73m^2 at least once in every patient. Total urinary protein excretion, β_2-microglobulinuria, and tubular proteinuria as determined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) showed pathological values in 18.9, 32.8 and 38.4 per cent of all 24 hour urine samples excreted during the days one to three after administration of high dose-MTX. This indicates a predominantly tubular type of proteinuria. Acute transient renal failure was seen only in one patient during one course of high dose-MTX treatment.

Introduction

Results of cytostatic therapy in osteosarcoma have been significantly improved in the last years by the use of high dose-MTX therapy [1]. According to current protocols the patients are treated initially with toxic doses of methotrexate (MTX) accompanied by leucovorin in combination with other cytostatic agents (Figure 1). In this way systemic toxicity of MTX usually can be avoided. However acute renal failure after administration of high dose-MTX remains a serious problem although nephrotoxicity can be reduced by administration of alkali. The present study differentiates tubular from glomerular nephrotoxic side effects of high dose-MTX treatment and evaluates the severity of the renal damage. Molecular weight dependent analysis of urinary proteins by SDS-PAGE was applied to detect changes in the pattern of urinary protein excretion [2].

Patients and methods

Details of the patients and the high dose-MTX treatment are given in Table I. High dose-MTX treatment was performed as outlined in the T7 protocol [1].

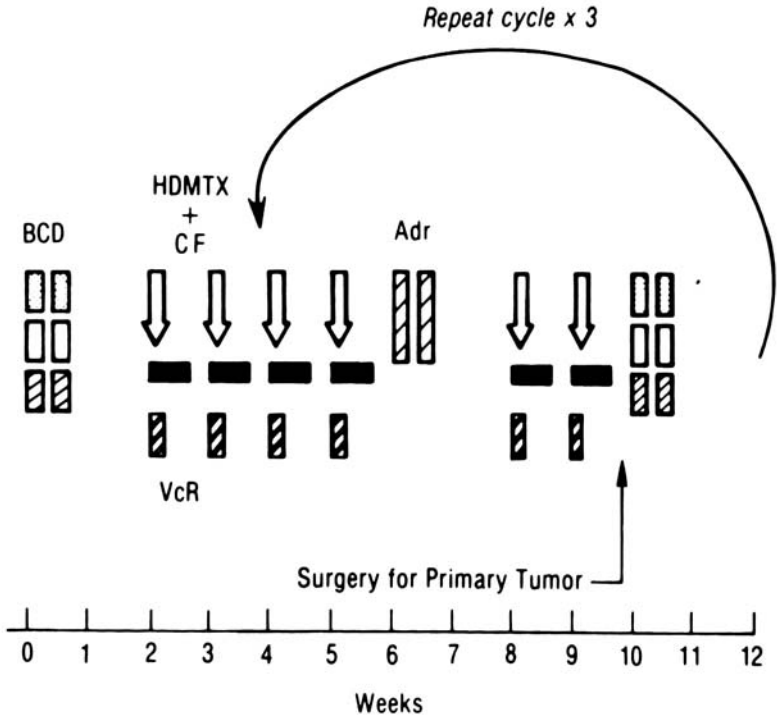

Figure 1. Cytostatic protocol used for treatment of osteosarcoma (T7 protocol according to Rosen [1]. Initially patients are treated by bleomycin (12mg/m^2), cyclophosphamide (600mg/m^2), and dactinomycin (450mg/m^2) (BCD) twice, followed by high dose-MTX (8–12g/m^2) and vincristine (VCR, 1.5mg/m^2) weekly as indicated, interrupted by a course of adriamycin (ADR, 45mg/m^2). Citrovorum factor (CF) is added as a rescue treatment. Eighteen courses of high dose-MTX are given over a period of nine months

Treatment was interrupted in patients two and three because of non compliance and in patient four because of therapeutic failure. Twenty-four hour urine collections were started at the time of MTX-infusion and continued for three to four days.

A urinary protein excretion >100mg/m^2/day was regarded as pathological. Urinary protein was measured according to the Biuret method. Normal excretion of β_2-microglobulin in healthy individuals is about 110μg/24hr/1.73m^2 with an upper limit of 300μg/24hr/1.73m^2 [3,4]. β_2-microglobulin was determined by ELISA (Pharmacial). SDS-PAGE was performed on 1.5mm thick slab gels with a linear acrylamide concentration of 10 per cent; the Laemmli buffer system [5] was used and the staining dye was Coomassie Blue G 250. The gels were scanned with the Desaga Quick Scan unit combined with an integration unit (Desaga, Heidelberg). The glomerulo-tubular protein ratio (GTPR) was calculated by dividing the total excretion of high molecular-weight (HMW) proteins with molecular weight above 68,000 daltons by that of low molecular weight (LMW) proteins with less than 68,000 daltons excluding the albumin peak. GTPR in 30 healthy subjects was always above 0.7, values below 1.0 correspond with

TABLE I. Data of patients and individual high dose-MTX treatment. On the right median values of C_{Cr}, TUP, β_2-microglobulinuria and GTPR are given. Significant changes from day 1 to another day are marked by *. (* = interval of confidence 95%, **99%)

Patient	Initials	Sex	Age (years)	Number of high dose-MTX courses	Mean single dose of MTX g/m²	Initial or relapse	C_{Cr} ml/min/1.73m²		TUP mg/24hr/m²	β_2-microglobulin excretion µg/24hr/1.73m²	GTPR
1	YE	F	9	18	10	initial	day 1	102.0	96.3	378.7	1.43
							day 2	111.5	66.5	259.5*	1.59
							day 3	122.0	95.6	273.0	2.07
							day 4	88.2	41.6**	224.9**	1.32
2	RB	M	22	14	9	relapse	day 1	99.2	44.5	155.7	0.77
							day 2	87.6	42.3	160.5	0.98
							day 3	98.8	57.8	154.8	1.10
3	PK	M	15	8	7	relapse	day 1	88.0	62.7	680.9	0.95
							day 2	115.0	71.6	292.9	1.06
							day 3	66.0	98.4	614.2	1.27
4	TW	M	8	12	9	initial	day 1	105.5	81.4	368.5	1.52
							day 2	93.4	54.3*	224.0*	1.23
							day 3	113.0	51.8	216.3	1.59
5	VK	M	17	18	10	initial	day 1	157.0	130.0	240.1	0.81
							day 2	158.0	71.5**	195.2	0.81
							day 3	197.5	79.1**	222.5	0.92

C_{Cr} = creatinine clearance; TUP = total urinary protein; GTPR = glomerulo-tubular protein ratio

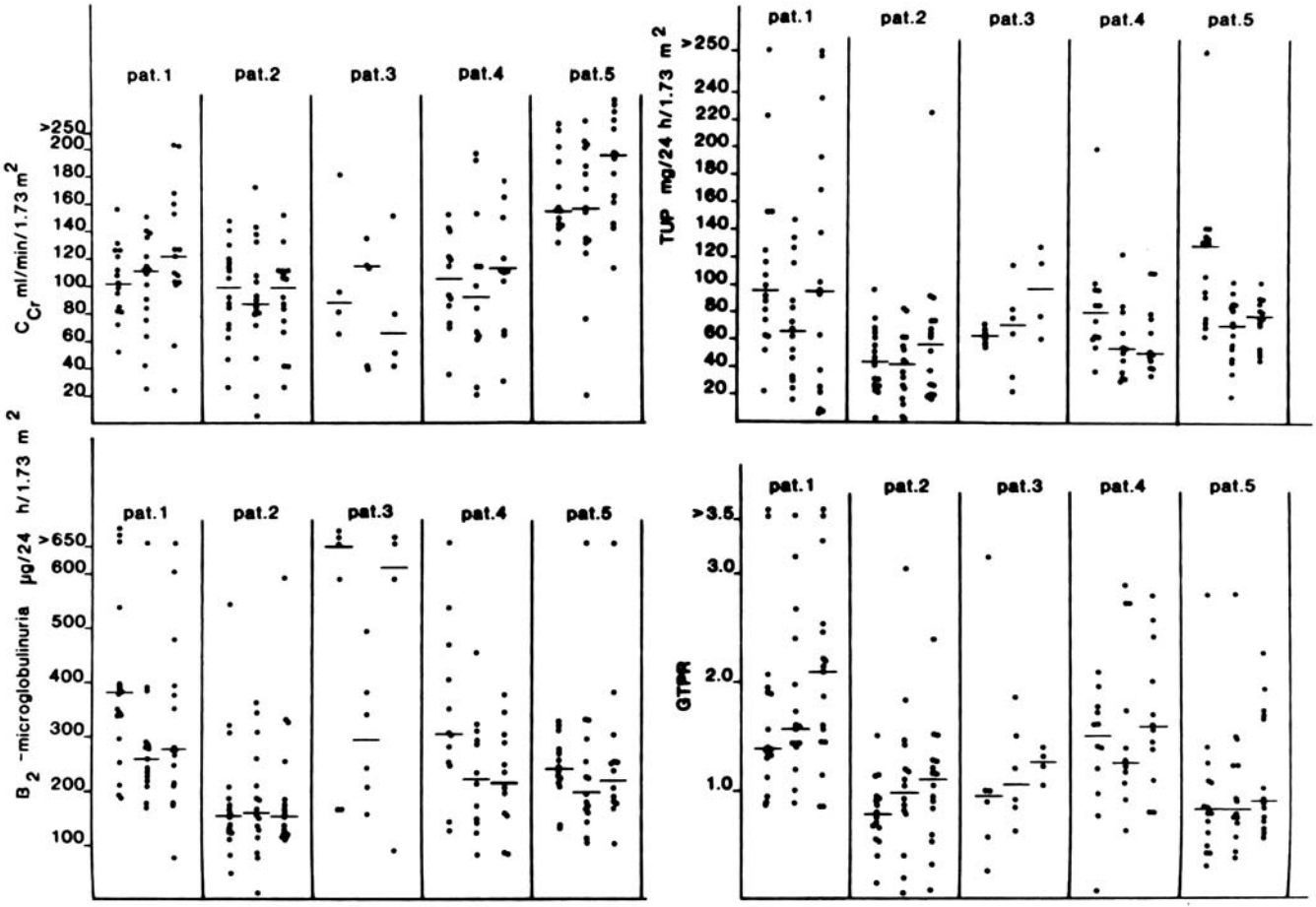

Figure 2. C_{Cr}, TUP, β_2-microglobulinuria and GTPR of each patient during the days 1–3(4) of each treatment course. The median values are marked. C_{Cr} = creatinine clearance; TUP = total urinary protein; GTPR = glomerulo-tubular protein ratio

LMW patterns in the gell [2]. For statistical analysis the Wilcoxon test was used.

Results

Individual results of creatinine clearance, urinary protein, β_2-microglobulinuria, and GTPR in the five patients are given in Table I and Figure 2. Usually pathological parameters became normal within 24–48 hours. However in each patient pathological values were seen not earlier than on day three. With increasing numbers of high dose-MTX treatment cycles in individual patients we could not detect any signs of cumulative nephrotoxicity. In each patient creatinine clearance dropped at least once to less than 40ml/min/1.73m^2. In 46 of 193 (23.8%) daily urine samples creatinine clearance was 80ml/min/1.73m^2. Urinary protein was >100mg/24hr/1.73m^2 in 38 of 201 (18.9%) samples and above 200mg/24hr/1.73m^2 in seven of 201 (3.5%). β_2-microglobulinuria was >300μg/24hr/1.73m^2 in 66 of 201 (32.8%) samples and GTPR was <1.0 in 78 of 203 (38.4%) samples. In 29 of 203 (14.3%) samples an increase of LMW proteinuria was found with GTPR values below 0.7. Increased β_2-microglobulinuria and tubular proteinuria as expressed by GTPR were more often seen in day one when compared with the following days. β_2-microglobulin was in 29 cycles in the pathological range on day one but only in 15 cycles on day two. GTPR was in 14 cycles in the pathological range on day one but only in eight cycles on day two. An acute symptomatic intoxication with transient renal failure was seen only once in patient two, who showed an increase of serum creatinine to 3mg/dl followed by a return to normal within several days.

Discussion

Although the nature of MTX nephrotoxicity is still unclear [6] our data indicate that a mixed pattern of glomerular and tubular lesions is the rule during administration of high dose-MTX for treatment of osteosarcoma with the protocol of cytostatic treatment mentioned above [1]. A transient decrease in creatinine clearance was seen in each of the patients. These results are in concordance with a recent study of nine adult patients with osteosarcoma and high dose-MTX treatment who showed a decrease of GFR to 43 per cent within 24–40 hours after drug administration [7]. Similar to our own observation the authors of this study failed to find a correlation between the number of high dose-MTX courses and renal toxicity. Urinary protein excretion was increased in 18.9 per cent of all treatment courses but was only of moderate degree. The results of β_2-microglobulinuria and SDS-PAGE suggest that most proteins excreted are of tubular origin. In 32.8 per cent of all urine samples investigated β_2-microglobulin was increased and in 38.4 per cent the electrophoretic pattern showed a predominant LMW pattern. The degree of tubular damage however seems to be rather moderate when compared with the profound reduction of creatinine clearance. A strict supervision of GFR during high dose-MTX therapy seems to be indispensable. We conclude, that in our patients treated according to the protocol mentioned above, nephrotoxic side effects were within tolerable limits.

Acknowledgments

We wish to acknowledge Dr H Pfueller for laboratory advice and Mrs I Krah for technical assistance.

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