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ARTICLE

Anthracycline Antibiotics Induce Acute Renal Tubular Toxicity in Children with Cancer

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Experimental evidence suggests that anthracyclines, widely used in cancer chemotherapy, may impair kidney function. We assessed kidney function by serum creatinine, urinary N-acetyl-β-D-glucosaminidase activity indices (NAGi) and microalbuminuria (MA) in 160 serum and urine samples obtained from 66 children with cancer. The effect of dexrazoxane was analyzed in 6 children on dexrazoxane supportive therapy in conjunction with daunorubicin (DNR) treatment, as compared with 6 children not receiving this agent. NAG_i was significantly ($p<0.05$) elevated after treatment by DNR, doxorubicin, epirubicin (EPI) and idarubicin (IDA). MA proved to be a less sensitive indicator of kidney damage than NAG_i. DNR resulted in a progressive deterioration of proximal tubular function as determined by linear regression analysis. The mean NAG_i in the dexrazoxane-treated group was significantly ($p<0.005$) lower than in children not receiving dexrazoxane prior to DNR treatment. In conclusion, our study demonstrated that DNR, EPI and IDA induced an acute renal tubular damage similar to known tubulotoxic agents as cisplatin, carboplatin, cyclophosphamide and ifosfamide. The damage was clinically mild and only a minor proportion of patients can be expected to develop long-lasting tubulopathy with negative impact on the quality of life. (Pathology Oncology Research Vol 13, No 3, 249–253)

Key words: nephrotoxicity, anthracycline therapy, dexrazoxane, NAGi, microalbuminuria

Introduction

Recent data indicated that daunorubicin (DNR) and doxorubicin (DXR) induce renal damage. Previously we have assessed acute glomerular impairment by cytostatic agents by measuring serum creatinine and cystatin C (cysC) levels as well as endogenous creatinine clearance. Our results indicated that platinum derivates and alkylating agents play a major role in cytostatics-induced glomerular toxicity, whereas anthracycline antibiotics did not impair glomerular function.[1] However, renal tubular dilation and increased excretion of lysosomal enzymes has been reported in the urine of rodents after treatment with anthracyclines.[2] In contrast to experimental animals, anthracycline–induced tubulotoxicity has not been clearly demonstrated in humans. Increased excretion of lysosomal enzymes, b2-microglobulin, and gross proteinuria due to renal damage have been reported in patients with ovarian cancer receiving sequential combination chemotherapy. These included DXR, cisplatin (CDDP) and cyclophosphamide (CYC).[3] In contrast, the Vienna Pediatric Oncology Group did not observe renal damage in children having been treated with high doses of CYC, DXR and vincristine (VCR) because of recurrent solid tumors.[4] Recently, some groups of investigators, including ours, have studied late nephrotoxicity in children with cancer in long-term remission.[5-7] These observations suggested that anthracycline antibiotics may have contributed to renal tubular damage in humans.

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Abbreviations

NAGi: N-acetyl-β-D-glucosaminidase activity normalized for urinary creatinine concentration, MA: microalbuminuria, DNR: daunorubicin, DXR: doxorubicin, CDDP: cisplatin, CARBO: carboplatin, CYC: cyclophosphamide, VCR: vincristine, EPI: epirubicin, IDA: idarubicin, IFO: ifosfamide, MHSCUD: Medical and Health Science Center of the University of Debrecen, HPOG: Hungarian Pediatric Oncology Group, ALL: acute lymphoblastic leukemia, NHL: non-Hodgkin lymphoma, SR: standard risk, IR: intermediate, HR: high risk, ECG: electrocardiography, SD: standard deviation, CARBO: carboplatin, IFO: ifosfamide, ASCO: American Society of Clinical Oncology

The aim of the present study was to evaluate renal function in children immediately before and after having received anthracycline antibiotics, i.e. DNR, DXR, epirubicin (EPI) and idarubicin (IDA). We have also investigated whether dexrazoxane, which has been shown to exert a potent cardioprotective effect in anthracycline-treated animals and human patients, may provide protection against the tubular toxicity of DNR.

Patients and methods

Patients

Renal function was evaluated in 160 serum and_urine samples obtained from 66 children with various tumors (*Table 1*). All were Caucasians, boys:girls=38:28, mean age: 8.6±5.5 years, age range: 0.5-25.0 years, median: 7.5 years, and all were treated at the Hematology/Oncology Unit of the Department of Pediatrics, Medical and Health Science Center of the University of Debrecen (MHSCUD), between October 1, 1999 and December 31, 2003. Samples were obtained immediately before and 24 hours after DNR, DXR, EPI and IDA treatment. All patients were treated according to standard oncology protocols of the Hungarian Pediatric Oncology Group (HPOG) (*Table 1*).[8-15]

Progressive damage caused by consecutive DNR treatment was examined in 26 patients with acute lymphoblastic leukemia (ALL) and non-Hodgkin's lymphoma (NHL) (boys:girls=20:6, all Caucasians, mean age: 8.7±4.5 years, age range: 2-18.0 years, median:7.0 years). Children with standard-risk (SR) ALL and NHL received 2, children with intermediate- (IR) and high-risk (HR) ALL received 4 courses of DNR, as indicated by the treatment protocol.[8]

Six additional children with ALL (boys:girls=4:2, mean age: 4.1±2.1 years, age range: 3.0-12.2 years, median 5.3 years) were admitted between January 1, 2000 and December 31, 2001, outside of the frames of the HPOG study. Their therapy strictly followed the original ALL BFM 95 protocol,[8] which does not include the compulsory application of dexrazoxane in conjunction with DNR and DXR administration. All other ALL patients were treated according to the HPOG-modified version of ALL BFM 95.This involved giving 300 mg/m^2 dexrazoxane prior to the use of either DNR or DXR. Prior to and after each anthracycline treatment, electrocardiography (ECG) and echocardiography was performed. Proximal tubular function of the 6 ALL patients receiving their anthracycline treatment without dexrazoxane prophylaxis was compared with that of 6 children (boys:girls=4:2, all Caucasians, mean age: 4.1±1.2 years, age range: 3.0-13.0 years, median 5.2 years) who did receive dexrazoxane before the application of DNR and DXR.

Informed consent was obtained from parents or responsible caretakers. The study was accepted by the Ethical Committee of MHSCUD.

Table 1. **Disease characteristics and treatment modalities of patients (n=66)**

Diagnosis	*Number of patients*	*Protocol*
Acute lymphoblastic leukemia	25	ALL-BFM 95[8]/ ALL IC-BFM 2002
Acute myeloid leukemia and myelodysplastic syndrome	6	AML-BFM 98[9]
Non-Hodgkin's lymphoma	8	NHL-BFM 95[10]
Hodgkin's lymphoma	13	DAL-HD[11]
Wilms' tumor	6	SIOP[12]
Bone tumors	2	COSS[13]
Soft tissue sarcoma	4	CWS[14]
Hepatoblastoma	2	PLADO[15]

Methods

Serum and urinary creatinine concentrations were determined by the kinetic Jaffe method and expressed in μmol/L.[16]

N-acetyl-β-D-glucosaminidase activity (NAG) was determined by the modification of the method of Horak et al., as described earlier, together with age-specific reference values. NAG activities were normalized for urinary creatinine concentration and given as NAG indices (NAG_i), expressed in μmol/min/mmol creatinine units.[17]

Microalbuminuria (MA) was determined in a 24-h collection sample by standard immunoturbidimetric method (Cobas Integra 400, Roche, Basel, Switzerland).

Standard urinalysis of a freshly void early morning midstream sample was performed according to standard methods at every checkup.

Statistical analysis

Descriptive statistics test (Statistica for Windows) was used to determine the normal distribution of the data. The results are reported as mean±standard deviation (SD). NAG_i and MA values were compared by one-way ANOVA. Critical differences between groups were assessed by paired t-test and by Newman-Keuls post-hoc test. In order to evaluate progressive changes in proximal tubular function in parallel with consecutive DNR treatment, post-treatment NAGi of individual patients were related to the first pretreatment NAGi of the respective patients considering the first value as 100%, and percentage changes were plotted against the number of treatment cycles. We fitted a line to the points, and the significant deference from the horizontal line was evaluated by linear regression analysis. Differences were regarded significant if $p<0.05$.

Results

As expected, there was no significant change in serum creatinine levels due to treatment with the various cytostatic agents (*Table 2*).

Mean NAG_i was significantly elevated ($p<0.05$) after DNR, IDA and EPI treatment (*Table 3*). Similar increase in NAG_i was observed after the use of agents with proven tubular toxicity, such as CDDP, carboplatin (CARBO), CYC and ifosfamide (IFO) (*Table 3*). The average amount of MA did not change significantly after anthracycline treatment, suggesting that MA is a less sensitive indicator of renal damage than NAG_i (*Table 3*).

The consecutive DNR courses were associated with an increasing posttreatment level of NAG excretion as expressed in the percentage of the pretreatment NAG_i (NAG%) of the individual patients. Linear regression analysis showed a significant ($p<0.05$) elevation. Mean posttreatment NAG% was three times as high after the fourth course as after the first course of DNR treatment (*Fig. 1*).

We studied the effect of dexrazoxane on DNR-induced excretion of NAG. The mean NAG_i in the dexrazoxane-treated group was significantly ($p<0.005$) lower (0.69±0.25 μmol/min/mmol) than in the group of patients not receiving dexrazoxane (1.79±1.45 μmol/min/mmol) in conjunction with DNR application. There was no effect of dexrazoxane treatment on DNR-induced MA (mean MA: 8.89±1.16 mg/L before treatment vs. 8.75±2.03 mg/L after treatment).

Table 2. **Effect of anthracycline treatment on serum creatinine concentration of patients**

Treatment (No. of patients)	*Pretreatment serum creatinine (mmol/L)*	*Posttreatment serum creatinine (mmol/L)*	*p*
CDDP (n=123)	56 ± 19	56 ± 19	0.96
CARBO (n=84)	50 ± 16	47 ± 12	0.74
CYC (n=251)	58 ± 27	54 ± 17	0.94
IFO (n=148)	58 ± 15	64 ± 22	0.99
DNR (n=150)	61 ± 15	59 ± 15	0.97
EPI (n=78)	53 ± 17	50 ± 14	0.98
IDA (n=46)	61 ± 14	54 ± 14	0.95
DXR (n=249)	60 ± 18	60 ± 18	0.90

CDDP: cisplatin, CARBO: carboplatin, CYC: cyclophosphamide, IFO: ifosfamide, DNR: daunorubicin, EPI: epirubicin, IDA: idarubicin, DXR: doxorubicin. Values are expressed as mean ± SD.

Discussion

As far as we know, acute renal tubulopathy caused by anthracycline derivatives has not been equivocally proven in humans. Our results proved that three of the studied anthracycline antibiotics induced a significant elevation in NAG-uria, DNR being the most toxic, followed by EPI and IDA. Interestingly, we did not find a significant elevation in NAG_i after DXR therapy in our patients, although renal tubular toxicity of this agent has been proven in rodents.[2] Since most of the patients receiving DXR in the course of their combined cytostatic therapy suffered from ALL and NHL, DXR was introduced in the postconsolidation phase of their treatment according to the applied protocols.[8] These patients were heavily pretreated with other tubulotoxic agents such as DNR and CYC (standard- (SR) and intermediate-risk (IR) ALL and NHL patients) and IFO (high risk (HR) ALL and NHL patients) during induction and intensification. This nephrotoxic pretreatment might have resulted in an already compromised tubular function that was not further deteriorated by DXR.

Indeed, a progressive increase in post-treatment excretion of NAG was demonstrated in children having received

Table 3. **Effect of cytostatic treatment on N-acetyl-b-D-glucosaminidase index (NAG_i) and microalbuminuria (MA) of patients**

Treatment (No. of patients)	*NAGi (mmol/min/mmol)*			*MA (mg/L)*		
	Pretreatment	*Posttreatment*	*p*	*Pretreatment*	*Posttreatment*	*p*
CDDP (n=123)	1.8 ± 1.9	7.6 ± 10.2	**0.007**	44.6 ± 42.5	66.9 ± 16.9	0.503
CARBO (No 84)	2.5 ± 3.3	4.5 ± 4.8	**0.007**	18.5 ± 13.6	32.3 ± 31.6	0.059
CYC (No 251)	2.9 ± 9.2	3.3 ± 4.7	**0.006**	22.3 ± 45.2	40.8 ± 80.2	**0.032**
IFO (No 148)	3.3 ± 3.6	8.4 ± 7.2	**0.006**	56.6 ± 11.2	182.5 ± 29.6	**0.014**
DNR (No 150)	2.2 ± 3.5	8.8 ± 9.5	**0.000**	9.9 ± 3.8	10.4 ± 3.3	0.632
EPI (No 78)	2.2 ± 3.5	8.4 ± 4.8	**0.002**	33.7 ± 26.5	44.1 ± 6.4	0.557
IDA (No 46)	2.5 ± 2.1	6.5 ± 5.2	**0.038**	11.3 ± 6.5	19.2 ± 17.5	0.261
DXR (No 249)	2.3 ± 2.4	2.7 ± 3.8	0.319	32.8 ± 65.9	44.3 ± 13.6	0.549

CDDP: cisplatin, CARBO: carboplatin, CYC: cyclophosphamide, IFO: ifosfamide, DNR: daunorubicin, EPI: epirubicin, IDA: idarubicin, DXR: doxorubicin. Values are expressed as mean ± SD.

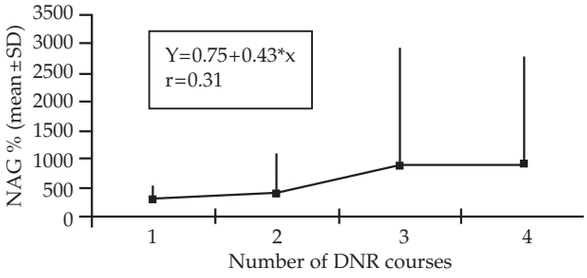

Figure 1. *Cumulative renal tubular toxicity of daunorubicin (DNR). Posttreatment NAG% elevated significantly ($p<0.05$) in association with consecutive DNR courses. Mean values are represented by black squares, bars indicate SD values.*

2 (SR patients) to 4 (IR and HR patients) courses of DNR during the induction phases of the applied ALL and NHL protocols. Prednisolone, vincristine, asparaginase and DNR represented potentially tubulotoxic agents of the induction treatment.

MA proved to be a less sensitive indicator of renal tubular damage than NAGi. In addition, MA, widely used to characterize renal tubular damage, cannot be considered an optimal marker of renal tubular function, since the level of MA is largely affected by impaired glomerular function, i.e. MA is not able to distinguish between renal glomerular and tubular damages. However, both the results of this study and our previous observations indicated that anthracycline antibiotics did not play a major role in glomerulotoxicity.[1]

The use of dexrazoxane to prevent cardiotoxicity in patients receiving anthracycline treatment has been recommended by the current guidelines of the American Society of Clinical Oncology (ASCO) only within the frames of clinical studies.[18] Recent experimental evidences demonstrated that anthracycline-induced nephrotoxicity can also be prevented by the use of dexrazoxane in rats.[19]. We took the opportunity to compare post-treatment NAG_i in a small cohort (n=6) of ALL patients receiving DNR treatment outside of the frame of the study of the Hungarian Pediatric Oncology Group (HPOG), i.e. not having received dexrazoxane prior to the use of DNR, with the proximal tubular function of sex- and age-matched ALL patients who were treated according to the ALL-BFM 95 protocol modified by the HPOG in the same period of time. These latter patients received 300 mg/m^2 dexrazoxane 30 minutes before DNR infusion (30 mg/m^2). We found significantly elevated NAG_i in children who did not receive dexrazoxane versus those who did, indicating that dexrazoxane can diminish renal tubular damage in humans.

In conclusion, our study demonstrated that DNR, EPI and IDA induced an acute renal tubular damage. The elevation of posttreatment NAGi after DNR and EPI treatment was similar to that observed after CDDP and IFO treatment. Impairment of tubular function, however, was obvious only by applying a sensitive laboratory method, such as determination of NAGi. The damage was clinically mild and only a minor proportion of patients can be expected to develop long-lasting tubulopathy with negative impact on quality of life.[7] We were able to detect significant tubuloprotective effect of dexrazoxane in association with DNR treatment. Because of the small number of patients involved, further studies are required to confirm the tubuloprotective effect of dexrazoxane in conjunction with anthracycline antibiotics.

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