Pharmacological Reports
2007, 59, suppl. 1, 205–209
ISSN 1734-1140

Busulfan treatment in patients before bone marrow transplantation and early indicators of renal tubular function

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Abstract:
In order to carry out safe anticancer chemotherapy, kidney function should be monitored. The aim of our work was to demonstrate the role of α_1-microglobulin (α_1-M) and N-acetyl-beta-D-glucosaminidase (NAG) for the monitoring of renal function in patients with hematological malignancies before and during high-dose busulfan-based conditioning regimen prior to bone marrow transplantation (BMT). On the basis of our study, we have shown that α_1-M concentrations and NAG activity increased in urine in patients with hematological cancers, especially after 13-dose busulfan therapy. Our preliminary results lead to the conclusion that both supportive therapy and administration of busulfan can impair tubular function. These observations have shown that it is necessary to carry out detail estimation of kidney excretory function during anticancer chemotherapy in the patients waiting for BMT. Detection of an increased α_1-M concentration and NAG activity in urine can be helpful for the recognition of the patients at high risk of tubular dysfunction. Disturbances of kidney function should be considered during the planning of individual drug dosage regimens.

Key words:
kidney function, α_1-M, NAG, busulfan

Introduction

Anticancer treatment often has to be limited or is precluded by kidney function disorders related to neoplastic disease or its chemotherapy. Therefore, it is necessary to carry out regular and comprehensive tests of renal function in cancer patients. They could be useful for the rapid medical intervention before the appearance of unwanted clinical symptoms [8, 15].

Because of their properties, a glycoprotein – α_1-microglobulin (α_1-M) and an enzyme – N-acetyl-beta-D-glucosaminidase (NAG) can be used as an early noninvasive indicators of proximal tubular cell damage. Tubular proteinuria occurs when glomerular function is normal, but the proximal tubules have diminished capacity to reabsorb and catabolize proteins, causing an increased urinary excretion of the low molecular weight proteins that normally pass through the

glomerulus, such α_1-M. Normal urine contains very small amounts of α_1-M. In conditions with disturbed tubular function, during diseases (chronic and acute pyelonephritis, kidney transplant rejection, Fanconi syndrome and Balkan nephropathy) and treatment with some drugs (aminoglycosides, cyclosporine), reabsorption of this protein is reduced and its increased amounts are found in urine [2, 18, 20–22, 26, 27, 29]. AG is a lysosomal enzyme located mainly in the renal proximal tubules. Due to its molecular weight (about 150,000), it does not normally pass through the glomerulus. It is physiologically excreted at low amounts in urine. Increased NAG enzymatic activity in urine has been found to be associated with various kidney injuries – arterial hypertension, diabetic nephropathy, renal transplant rejection) and is a sign of drug nephrotoxicity (cisplatinum, ifosfamide) [3, 6, 7, 10, 24]. The increased urinary excretion of these markers often precede clinical signs of renal dysfunction by several days.

The purpose of the present study was to establish the role of a_1-M and NAG for the monitoring of renal impairment in patients with hematological malignancies before and during high-dose busulfan-based conditioning regimen prior to bone marrow transplantation.

Materials and Methods

Studies were carried out in 131 persons: 22 patients before bone marrow transplantation (8 – with chronic myelogenous leukemia (CML), 10 – with acute myeloblastic leukemia (AML), 3 – with acute lymphoblastic leukemia (ALL), 1 – with osteomyelofibrosis) and 65 healthy volunteers as a control group for NAG deteremination and 44 healthy volunteers as a control group for α_1-M deteremination (Tab. 1).

Renal excretory function (α_1-M, creatinine concentrations and NAG activity in urine) was determined before and after 1, 9 and 13 doses of conditioning busulfan therapy. Busulfan was administered orally at a dose of 16 mg/kg b.m. each day for 4 days before BMT. Before and during cytostatic chemotherapy, patients received ciprofloxacine 500 mg × 2 daily doses, trimethoprim/sulfamethoxasole 960 mg × 2 weekly doses, antibiotics according to the result of bacteriological culture (cefotaxim 1000 mg × 3 daily doses, amoxicillin/clavulanic acid 1200 mg × 3 daily doses, ceftazidim 1000 mg × 2 daily doses, meropenem 1000 mg × 3 daily doses, vancomycin 1000 mg × 2 daily doses, imipenem/cilastin 500 mg × 3 daily doses), ketoconazole or fluconazole 200 mg × 2 daily doses and acyclovir 200 mg × 5 daily doses.

α_1-M and NAG were analyzed in the second morning spot urine sample. The enzyme-linked immunosorbent assay (ELISA) was used for the quantitative determination of α_1-M concentration. Detection limit of the method is 0.006 mg/l, according to the assay kit producer (Immundiagnostik AG). The activity of NAG was determined by the enzymatic method, using p-nitrophenyl-N-acetyl-β-D-glucosamide as a substrate [30]. Released p-nitrophenol (NAG catalyzes the hydrolysis of p-nitrophenyl-N-acetyl-β-D-glucosamide into p-nitrophenol and N-acetylglucosamine), proportional to the enzymatic activity, was determined colorimetrically at 410 nm. Detection limit of the method was 1.6 U/l. The influence of diuresis volume on protein concentration and enzyme activity was eliminated by expressing α_1-M concentration and NAG activity in relation to creatinine concentration in urine [3, 4, 14, 18, 24]. Creatinine concentrations in urine were determined according to Jaffe's colorimetric method.

Statistical comparison between the patient groups was performed with nonparametric Mann-Whitney test. A probability of less than 0.05 was considered statistically significant. The protocol for the study

Tab. 1. Characteristics of patients and healthy persons as a control group

Group	Number of persons	Age (years)		Body weight (kg)	
		$\overline{X}$	± SD	$\overline{X}$	± SD
Healthy volunteers					
N-acetyl-beta-D-glucosaminidase (NAG)	65	36.2	8.9	63.2	8.5
α_1-microglobulin (α_1-M)	44	40.1	9.9	68.8	13.8
Patients with hematological cancer	22	38.0	11.7	70.6	13.4

was approved by the Ethics Committee of Wrocław Medical University.

Results

In our study, we observed statistically significant increase in α_1-M concentration in a group of patients before and during myeloablative treatment in comparison with the group of healthy volunteers. The mean values of α_1-M concentrations in healthy persons, patients before and during conditioning therapy with busulfan are shown in Table 2. Statistically significant differences in the protein concentration between patients before anticancer treatment and healthy persons point to the decrease in kidney tubular function caused by the disease and previous supportive treatment with some drugs (acyclovir, vancomycin, quinolones, cefalosporine derivatives, cotrimoxazole) used before conditioning therapy.

The comparison of the mean values of NAG activity between patient groups before and during busulfan therapy, and healthy volunteers is shown in Table 3.

In the examined groups of patients, the concentration of creatinine in urine differed significantly ($p < 0.05$), regarding the values obtained after the 1st dose of busulfan (0.84 ± 0.83 g/l), 9th (0.96 ± 0.61 g/l), 13th (0.81 ± 0.51 g/l), when compared to this parameter in healthy persons, reaching 1.28 ± 0.39 g/l.

Discussion

Kidney diseases frequently complicate malignancy and its treatment. The spectrum of insufficiency of the main organ responsible for drug excretion includes acute or chronic renal failure, and tubular disorders. This problem can be very important in transplant patients.

Tab. 2. Concentration of α_1-microglobulin (α_1-M) in patients before and during the conditioning regimen with busulfan and in healthy persons as a control group

Group	Number of persons	α_1-M concentration/α_1-M/creatinine [mg/g creatinine]		Percent changes in α_1-M concentration
		$\bar{X}$	± SD	
Healthy volunteers	44	0.74	0.97	
Patients before busulfan therapy	17	1.04*	0.71	40.5
Patients after 1st dose of busulfan	19	1.39*	0.91	87.5
Patients after 9th dose of busulfan	18	2.61*	2.60	252.7
Patients after 13th dose of busulfan	17	4.00*	4.07	440.5

Statistically significant difference in the mean values of α_1-M concentration between healthy volunteers and patients before and during cytostatic treatment:* $p < 0.05$ *vs.* control group

Tab. 3. Activity of N-acetyl-beta-D-glucosaminidase (NAG) in patients before and during busulfan chemotherapy and in healthy persons as a control group

Group	Number of persons	NAG activity/NAG/creatinine [U/g creatinine]		Percent changes in NAG activity
		$\bar{X}$	± SD	
Healthy volunteers	65	3.52	1.99	
Patients before busulfan therapy	18	2.86	2.47	–18.8
Patients after 1st dose of busulfan	20	2.99	1.61	–15.1
Patients after 9th dose of busulfan	19	3.92	3.04	11.4
Patients after 13th dose of busulfan	18	4.88	5.77	38.6

During our study, we estimated the tubular function in patients with various hematological diseases, submitted to BMT during the ablative treatment with busulfan. This drug is primarily eliminated by conjugation with glutathione, which is catalyzed by glutathione S-transferase (GST) A1-1, a major GST isoform in the liver and kidney. This cytostatic agent was the most seriously toxic to the liver, lungs and nervous system. Hyperuricemia or uric acid nephropathy occurs during initial treatment of patients with leukemia. In bone marrow transplant patients, the dose of busulfan that is given in combination with other drugs can also cause nephrotoxicity prior to transplantation [15, 23, 25].

The population of healthy volunteers was used as the reference for comparison with the clinical material. In healthy persons (females and males jointly), α_1-M concentrations and NAG activity in urine did not differ from the values reported in the literature by other authors [1, 9, 11–13, 19, 29].

Available literature lacks sufficient data concerning the changes in the above-mentioned early indicators of renal tubular damage in patients during busulfan-based conditioning therapy. Among these two parameters, especially α_1-M concentrations in urine differed statistically significantly in patients in comparison to the control group of healthy persons. Changes in the urinary NAG activity were not so significant as α_1-M but after 9th and 13th dose of busulfan its activity has slowly increased.

The obtained results are similar to those reported by other authors, who observed increased protein concentration and enzyme activity in patients with leukemia, myelodysplastic syndromes, solid tumors [12, 28, 30]. Severini et al. reported an increased serum NAG activity in patients with breast, stomach and liver cancers. It can probably be accompanied by increased activity of the enzyme in urine [21]. Some authors observed about 54% increase in α_1-M concentration and 66% rise in NAG activity in BMT patients. They noted elevation of these parameters by 95% and 98% after the myeloablative therapy [16].

Patzer et al. reported the increased α_1-M concentration in 18/40 patients before myeloablative therapy and in 16/40 patients 1 year after BMT, in 13/33 at 2 years in patients receiving busulfan and other anticancer drugs (cyclophosphamide, melphalan, etoposide, thiotepa or antithymocyte globulin). NAG activity in this group of patients was the highest before BMT but after 1 year it fell down significantly and remained stable until 2 years [17].

In the kidney, ultrastructural metabolic and biochemical changes can be most often observed in S3 segment of proximal tubules. A decrease in ATP quantity, increase in intracellular concentration of calcium, as well as activation of phospholipases have been observed in the cells with insufficient amount of oxygen. Those processes can lead to the damage of cell membrane and increase the release of proteins and enzymes, including α_1-M, NAG, into the renal tubules. Another cause of the observed enzymuria can be the loss of integrity of cellular membrane in the renal tubules which results from peroxidation of membrane phospholipids through oxygen free radical and the products of their oxidation, occurring at higher amount in the process of oncogenesis. These changes can lead to hemodynamic disorders [5].

On the basis of this study results, it is concluded that impaired tubular function provoked by supportive treatment and chemotherapy with busulfan can be dangerous and might be the cause of failure in therapy. In patients before BMT treated with busulfan, regular determination especially of urinary α_1-M concentration can be helpful for the recognition of the patients at high risk of tubular dysfunction. Disturbances of kidney function should be considered during the planning of individual drug dosage regimens.

References:

1. Altunbas S, Yildiz D, Anarat A, Refik Burgut H: Renal tubular dysfunction in epileptic children on valproic acid therapy. Pediatr Nephrol, 2001, 16, 256–259.
2. Borzym-Kluczyk M, Darewicz B, Knaś M, Szajda SD, Sulik M, Olszewska E, Zwierz K: The activity of N-acetyl-β-D-glucosaminidase and its isoenzymes in the renal tissue, serum and urine of patients with renal cancer. Współcz Onkol, 2005, 9, 287–290.
3. Brzóska MM, Stypułkowska A, Zwierz K, Moniuszko-Jakoniuk J: Urinary activities of N-acetyl-β-D-glucosaminidase and its isoenzyme B in cadmium-exposed rats. Pol J Environ Stud, 2004, 13, 121–125.
4. Chatterjee S, Velicer LF, Sweeley CC: Glycosphingolipid glycosyl hydrolases and glycosidases of synchronized human KB cells. J Biol Chem, 1975, 250, 4975–4985.
5. Cobos E, Hall RR: Effects of chemotherapy on the kidney. Semin Nephrol, 1993, 13, 297–305.
6. Duława A, Bułdak Ł, Krysiak R, Okopień B: Hormonal supplementation in endocrine dysfunction in critically ill patients. Pharmacol Rep, 2007, 59, 139–149.

7. Humphreys BD, Soiffer RJ, Magee CC: Renal failure associated with cancer and its treatment: an update. J Am Soc Nephrol, 2005, 16, 151–161.
8. Kintzel PE, Dorr RT: Anticancer drug renal toxicity and elimination: dosing guidelines for altered renal function. Cancer Treat Rev, 1995, 21, 33–64.
9. Korinthenberg R, Wehrle L, Zimmerhackl LB: Renal tubular dysfunction following treatment with anti-epileptic drugs. Eur J Ped, 1994, 11, 855–858.
10. Kröning R, Katz D, Lichtenstein AK, Nagami GT: Differential effects of cisplatin in proximal and distal renal tubule epithelial cell lines. Br J Cancer, 1999, 79, 293–299.
11. Marks SD, Shah V, Pilkington C, Woo P, Dillon J: Renal tubular dysfunction in children with systemic lupus erythematosus. Pediatr Nephrol, 2004, 3, 141–148.
12. Moriguchi J, Ezaki T, Tsukahara T, Furuki K, Fukui Y: Comparative evaluation of four urinary tubular dysfunction markers, with special references to the effects of aging and correction for creatinine concentration. Toxicol Lett, 2003, 143, 279–290.
13. Mukhopadhyay B, Shashikant C, Lobo V, Gang S: Enzymuria pattern in early post renal transplant period: diagnostic usefulness in graft dysfunction. In J Clin Biochem, 2004, 19, 14–19.
14. Noto A, Yasunao O, Sachio M, Mitsuru Y: Simple, rapid spectrophotometry of urinary N-acetyl-β-D--glucosaminidase with use of new chromogenic substrate. Clin Chem, 1983, 29/10, 1713–1716.
15. Orzechowska-Juzwenko K: Chemotherapy of organ and systemic neoplasms (Polish). Volumed Scientific Publishers Ltd., Wrocław, 2000.
16. Patzer L, Kentouche K, Ringelmann F, Misselwitz J: Renal function following hematological stem cell transplantation in childhood. Pediatr Nephrol, 2003, 18, 623–635.
17. Patzer L, Ringelmann F, Kentouche K, Fuchs D: Renal function in long-term survivors of stem cell transplantation in childhood. Bone Marrow Transpl, 2001, 3, 319–327.
18. Penders J, Delanghe JR: Alpha 1-microglobulin: Clinical laboratory aspects and applications. Clin Chim Acta, 2004, 346, 107–118.
19. Pless-Mulloli T, Boettcher M, Steiner M, Berger J: Alpha-1-microglobulin epidemiological indicator for tubular dysfunction induced by cadmium. Occup Environ Med, 1998, 55, 440–445.
20. Price RG: The role of NAG (N-acetyl-β-D-glucosaminidase) in the diagnosis of kidney disease including the monitoring of nephrotoxicity. Clin Nephrol, 1992, 38, Suppl 1, 14–19.
21. Severini G, Diana L, Di Giovannandrea R, Tirelli C: A study of serum glycosidases in cancer. J Cancer Res Clin Oncol, 1995, 121, 61–63.
22. Skrezek C, Bertermann H, Schulz FP, Konig B: NAG (N-acetyl-beta-D-glucosaminidase) – a sensitive marker for disorders of kidney function. Urologe A, 1990, 29, 27–31.
23. Stolarska M, Mlynarski W, Zalewska-Szewczyk B, Bodalski J: Cytoprotective effect of amifostine in the treatment of childhood neoplastic diseases – a clinical study including the pharmacoeconomic analysis. Pharmacol Rep, 2006, 58, 30–34.
24. Tylicki L, Biedunkiewicz B, Chamienia A, Wojnarowski K, Zdrojewski Z, Aleksandrowicz E, Lysiak-Szydlowska W, Rutkowski B: Renal allograft protection with angiotensin II type 1 receptor antagonists. Am J Transplant, 2007, 7, 243–248.
25. Ullery LL, Gibbs JP, Ames GW, Senecal FM, Slattery JT: Busulfan clearance in renal failure and hemodialysis. Bone Marrow Transpl, 2000, 25, 201–203.
26. Verplanke AJW, Herber RFM, de Wit R, Veenhof CHN: Comparison of renal parameters in the assessment of cis-platin induced nephrotoxicity. Nephron, 1994, 66, 267–272.
27. Wiela-Hojeńska A, Orzechowska-Juzwenko K: N-acetyl-β-D-glucosaminidase and its clinical significance (Polish). Pol Arch Med Wewn, 1999, 101, 359–366.
28. Wiela-Hojeńska A, Orzechowska-Juzwenko K, Świerkot J, Wiland P, Hurkacz M, Szechiński J: Monitoring methotrexate therapy in patients with rheumatoid arthritis. Int J Clin Pharmacol Therap, 2004, 42, 434–441.
29. Yu H, Yanagisawa Y, Forbes MA, Cooper EH, Crokson RA, MacLennan IC: Alpha-1-microglobulin: an indicator protein for renal tubular function. J Clin Pathol, 1983, 36, 253–259.
30. Zwierz K, Grudzienski A, Głowacka D: N-acetyl-β-D-glucosaminidase activity in urine. Acta Med Acad Sci Hung, 1981, 38, 145–150.

Received:
March 28, 2007; in revised form: July 23, 2007.