Urinary Activities of N-acetyl-β-D-glucosaminidase and Its Isoenzyme B in Cadmium-Exposed Rats

M. M. Brzóska¹, A. Stypułkowska², K. Zwierz²*, J. Moniuszko-Jakoniuk¹

¹Department of Toxicology, Medical University of Białystok, Mickiewicza 2C, 15-222 Białystok, Poland
²Department of Pharmaceutical Biochemistry, Medical University of Białystok, Mickiewicza 2A, 15-222, Białystok, Poland

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Abstract

This paper aims to assess the relationship between the urinary activity of N-acetyl-β-D-glucosaminidase (NAG) and its isoenzyme B (NAG-B) and cadmium (Cd) concentration in the urine as well as to evaluate which of these lysosomal enzymes may be a more useful biomarker for the monitoring of Cd-induced tubular damage. For this purpose we have used an experimental model in rats chronically exposed to Cd in which we noted damage to the proximal tubules, including lysosomes. In rats intoxicated with 5 mg Cd/dm³, the urinary activities of NAG and NAG-B increased after 12 weeks of treatment, while at 50 mg Cd/dm³ - activities increased already after the 1st week. The urinary Cd excretion in Cd-exposed rats, but not in the non-exposed ones, positively correlated with the activities of NAG and NAG-B. A positive correlation which was observed between NAG and NAG-B activities was stronger in Cd-exposed animals than in those not exposed. The results of the present and our previous histopathological and histoenzymatic studies, confirm the usefulness of total NAG and NAG-B as sensitive markers of proximal tubular injury for the monitoring of chronic exposure to Cd. Taking into account the strong correlation between the total NAG and its isoenzyme B, similar correlation coefficients between their activities and Cd concentration in urine and simplicity of total NAG determination compared to that of NAG-B, one can conclude that the determination of total NAG is suitable for the monitoring of exposure to Cd. As the urinary activity of total NAG is a sum of activities of several isoenzymes, which may be influenced by various factors, and the intralysosomal localization of NAG-B, we hypothesize that NAG-B should be recommended as a sensitive and useful marker for routine monitoring of chronic exposure to Cd.

Keywords: cadmium, nephrotoxicity, urinary markers, N-acetyl-β-D-glucosaminidase, isoenzyme B of N-acetyl-β-D-glucosaminidase

Introduction

The kidney has been considered the critical organ for cadmium (Cd) toxicity following long-term exposure in humans and experimental animals. Cd-induced kidney injury is characterized first of all by proximal tubular dysfunction believed to be irreversible at advanced stages [1, 2, 3, 4]. Recent epidemiological data indicate that tubular dysfunction may appear in environmentally exposed subjects at lower levels of exposure to Cd than previously anticipated [5, 6]. Our own experimental studies [7] also revealed that Cd can damage the kidney, especially the main tubules (proximal convoluted tubules and straight tubules), at relatively low exposure and low accumulation in this organ. Thus it is of special importance to monitor the exposure to Cd using sensitive biomarkers allowing detection of subclinical changes at the stage when they are still reversible [4, 8-10].

At present, various biomarkers are used for the detection of changes in the kidney function under Cd influence. They involve functional markers (creatinine and β₂-microglobuline in serum, low and high molecular weight pro-
teins and calcium in urine), cytotoxicity markers (tubular antigens and enzymes in urine) and biochemical markers (glycosaminoglycans, kallikrein, sialic acids, eicosanoids in urine). The main indices allowing detection of early effect of Cd on the proximal tubules are enzymes, kidney antigens and low molecular weight proteins [10, 11].

N-acetyl-β-D-glucosaminidase (NAG), a lysosomal enzyme located mainly in the proximal tubules, and especially its isoenzyme B (NAG-B) have been recognized as the most sensitive indicators of the kidney proximal tubular injury [5, 6, 9, 12]. But until now the total activity of NAG is mainly measured for the monitoring of environmental and occupational exposure to Cd, rather than that of NAG-B [11, 13].

Since NAG-B is the lesional form of NAG, we have checked whether or not the activity of this isoenzyme may be a more useful biomarker for the monitoring of exposure to Cd. For this purpose we have created an experimental model in rats, in which we noted damage to the proximal tubules, including lysosomes [7, unpublished data]. In this model, we have assessed the relationship between urinary activities of total NAG and NAG-B, and between each of these enzymatic markers of proximal tubular damage and urinary Cd excretion.

Materials and Methods

Animals

Thirty-six inbred adult (2-month-old) male albino rats (Wistar strain) of initial body weight 180-200 g were used. Since birth and during the whole course of the experiment, the animals were housed in an environmentally controlled animal house, at a temperature of 22 ± 1°C, with a relative humidity of 50 ± 10% and a 12-hr light/dark cycle. They were allowed free access to drinking water and a standard granulated rodent laboratory LSM chow (Fodder Manufactures, Motycz, Poland). Cd concentration in the diet was assessed in our laboratory to be 0.211 mg/kg.

Experimental Design

The rats were randomly allocated to three groups, with twelve animals in each group. Two groups of rats received an aqueous solution of CdCl₂ at the concentration of 5 or 50 mg Cd/dm³ as the only drinking fluid for 24 weeks. Control rats drank redistilled water uncontaminated with Cd. Before the beginning of the experiment and after each consecutive week of its duration, 24-hour urine was collected in glass metabolic cages. Cd concentration and activities of total NAG and NAG-B were determined in the urine samples.

The study was approved by the Local Ethics Committee for animal experiments in Bialystok. Procedures involving the animals and their care conformed to the institutional guidelines, in compliance with national and international laws and Guidelines for the Use of Animals in Biomedical Research [14].

Cd Analysis

Cd concentration in the urine samples, diluted with ultra-pure water (Milli-Q system, Millipore Corporation, USA), was determined by flameless atomic absorption spectrometry method (Atomic Absorption Spectrophotometer Z-5000, Hitachi, Japan) with electrothermal atomization in a graphite furnace and automatic dosage as reported previously [7, 15]. To calibration, standard solution of Cd (Sigma, USA) assigned for atomic absorption spectrometry was used. Internal quality control was employed to keep the measurement process reliable.

Determination of NAG and NAG-B

The activities of NAG and NAG-B in the urine were determined according to Zwierz et al. [16], using p-nitrophenyl-N-acetyl-β-D-glucosamidase (Sigma, USA) as substrate. Released p-nitrophenol (NAG catalyzes the hydrolysis of p-nitrophenyl-N-acetyl-β-D-glucosamidase into p-nitrophenol and N-acetylglycosamine), proportional to the enzymatic activity, was determined colorimetrically at 410 nm using a Hitachi U-3010 spectrophotometer. Heat stable NAG-B was determined after 3-hour preincubation of samples without substrate at 50°C, and 1-hour at 37°C with the substrate.

Determination of Creatinine

To normalize Cd concentration, urinary creatinine was determined colorimetrically (Hitachi U-3010 spectrophotometer) according to Jaffe’s reaction using a diagnostic laboratory test (POCH, Poland).

Statistical Analysis

A one-way analysis of variance (ANOVA) was used to estimate statistical differences. A linear Pearson’s correlation was performed to evaluate the relationship between Cd concentrations and the activities of NAG and NAG-B. P values < 0.05 were considered significant.

Results

The urinary excretion of Cd in the control animals was low during the whole experiment and achieved values below or about 1 μg/g creatinine (ranged from 0.606 to 1.426 μg/g creatinine). In the rats drinking aqueous solutions of CdCl₂, the urinary excretion of Cd varied depending on the level of exposure and its duration. At exposure to 5 mg Cd/dm³, Cd concentration in the urine ranged from 2.264 to 10.968 μg/g creatinine whereas at 50 mg Cd/dm³ - from 8.104 to 26.763 μg/g creatinine.

The urinary activities of NAG and NAG-B increased dose- and time-dependently as a result of exposure to Cd (Fig. 1). In rats exposed to 5 mg Cd/dm³, starting from the 12th week, NAG and its isoenzyme B activities were statistically significantly enhanced (from 1.5 to 2.8-fold...
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Fig. 1. Urinary NAG and NAG-B activities in control and Cd-exposed rats. * - statistically significant (p < 0.05) difference to the control group was noted from the 12th to the 24th wk; † - statistically significant difference to the control and 5 mg Cd/dm³ groups was noted from the 1st to the 24th wk.

depending on exposure duration) compared to the control and remained at similar level to the end of the experiment (Fig. 1). In the animals treated with 50 mg Cd/dm³, a marked increase in both enzyme activities in comparison to the control (from 2.1 to 6.3 times, depending on the exposure duration) and 5 mg Cd/dm³ groups was observed already after the 1st week and it remained until the last week of exposure (Fig. 1).

In both Cd-exposed and non-exposed (control) rats, there was a high positive correlation between urinary activities of NAG and its isoenzyme B, but in the animals treated with Cd the coefficient was higher than in control (Fig. 2). In the rats not exposed to Cd, the urinary excretion of this toxic metal did not correlate with NAG and NAG-B activities, whereas in those exposed to Cd a high positive correlation was observed between the urinary Cd concentration and the activities of NAG and NAG-B (Figs. 3 and 4).

Discussion

The aim of the present study was to assess the relationship between the urinary activity of total NAG and its isoenzyme B and between the both Cd concentrations in the urine. Moreover, we intended to evaluate which of these lysosomal enzymes may be a more useful biomarker for the monitoring of Cd-induced kidney tubular damage. For this purpose, we determined urinary Cd concentrations and activities of NAG and NAG-B and analyzed correlations between these variables in the experimental model of rats’ exposure to Cd leading to the kidney tubular damage [7]. The results concerning urinary activities of these enzymatic markers of renal tubular injury we compared with our other results related to the effect of Cd on kidney status. Based on biochemical, histopathological, histoenzymatic and immunocytochemical studies, we have noted that Cd at the used levels of exposure (5 and 50 mg Cd/dm³) dose- and time-dependently damaged the

Fig. 2. Relationship between urinary NAG and NAG-B activities in control and Cd-exposed rats.

Fig. 3. Relationship between urinary Cd concentration and NAG activity in control and Cd-exposed rats.
ions in the organ. A small quantity of ions (released from ions, released into tubular lumen, ions released are transported to the kidney, and retains Cd in the kidney for a long time. Part of the Cd-degraded. The free Cd-tubular cells, and is transported into lysosomes where it is pinocytotic vesicles in the brush border of the proximal tubular membranes and next into extracellular space via damaged lysosomal excretion of both enzymes under Cd influence resulted in the main tubules, including damage to the lysosomes and reticulum. NAG-B is an intralysosomal membrane-bound enzyme, which is released into urine when disruption of lysosomal membranes occurs. It has been reported as a highly sensitive indicator of Cd-induced tubular toxicity and thus was recommended for the biological monitoring of exposure to this heavy metal [8-10, 12, 22].

Apart from NAG and its isoenzyme B, acid phosphatase (AcP) is also an enzyme marking lysosomes and lysosomal membranes. It has been recognized, together with NAG and NAG-B, as an early marker of cytotoxic action of Cd in the main tubules. Histochemical evaluation of activity and location of AcP allow detecting lysosomal damage at an early stage. We have noted that Cd, at both levels of exposure, damaged several structures in the kidney cells, including lysosomes and the cytotoxic action of Cd located mainly in the main tubules.

The increased urinary activities of NAG and NAG-B in rats exposed to Cd are well correlated with early lesions in the main tubules, including damage to the lysosomes (as observed based on the histochemical reaction for AcP) noted in these animals and considerably preceded the occurrence of changes in other biochemical markers of kidney status [7, unpublished data]. The enhanced excretion of both enzymes under Cd influence resulted from their leakage into cytoplasm via damaged lysosomal membranes and next into extracellular space via damaged cellular membranes.

A positive correlation between Cd excretion and urinary NAG and NAG-B activities has been also reported in humans exposed to Cd from environmental sources and in Cd-workers [9, 12].

In summary, the results of this and our other studies [7, unpublished data] confirm the usefulness of total NAG and NAG-B as sensitive markers of proximal tubular injury for the monitoring of chronic exposure to Cd. Taking into account the strong correlation between total NAG and its isoenzyme B, similar correlation coefficients between their activities and Cd concentration in the urine and simplicity of total NAG determination compared to that of NAG-B, one can conclude that determination of total NAG is suitable for the monitoring of exposure to Cd. But, as the urinary activity of total NAG is a sensitive and useful marker for the monitoring of chronic exposure to Cd.
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References