Bull. Environ. Contam. Toxicol. (2004) 73:437–442

DOI: 10.1007/s00128-004-0448-3

Bulletin of Environmental Contamination and Toxicology

Effect of Cadmium on Glycosaminoglycans in the Bone of Rats

K. Średzińska,[1] A. Galicka,[1] M. M. Brzóska,[2] A. Gindzieński[1]

[1] Department of Medical Chemistry, Medical University of Bialystok, 15-230 Bialystok 8, Poland
[2] Department of Toxicology, Medical University of Bialystok, 15-230 Bialystok 8, Poland

Received: 9 April 2004/Accepted: 15 July 2004

Cadmium (Cd), one of the most toxic heavy metals, is an environmental and occupational pollutant that creates a serious threat for living organisms. In addition to kidney damage, long term exposure to this metal leads to bone injury (Järup 2002; Wang et al. 2003). Osteomalacia and/or osteoporosis with increased prevalence of bone fractures, have been reported in humans and experimental animals chronically exposed to Cd (Katsuta et al. 1994; Brzóska et al. 2001; Wang et al. 2003). However, the mechanisms of the damaging action of Cd in bone have still not been fully clarified (Kjellström 1992). It has been reported that Cd has both direct and indirect effects on bone metabolism causing a decrease in both mineral and matrix content (Miyahara et al. 1980; Iguchi and Sano 1982).

The bone matrix mainly consists of type I collagen and glicosaminoglycans (GAGs), which are usually found covalently linked to protein in the form of proteoglycans (PGs). GAGs are linear polymers of repeated disaccharides, in most cases containing an O-sulphated N-acetylhexosamine and a hexuronic acid. The most common forms of GAGs are chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), heparin (H), keratan sulfate (KS), and hyaluronic acid (HA) (Beaty et al. 1987). PGs are present mainly on surfaces, in basement membranes and in extracellular matrix (ECM) that occupies the intercellular spaces in various tissues. Cell specific growth factors and enzymes immobilized in ECM and at the cell surface are bound to GAGs. PGs and GAGs have been shown to regulate protein secretion and gene expression in certain tissues by mechanisms involving both membrane and nuclear events, including the binding of GAGs to transcription factors. It has been observed in developing vertebrate that marked changes in PGs expression occur, suggesting that these macromolecules play a role in cell differentiation (Beaty et al. 1987; Hardingham and Fosang 1992).

Previously, we have noted that exposure of rats to Cd at concentrations of 1, 5 and 50 mg Cd/L, leads to bone demineralisation and weakening in their mechanical properties (Brzóska et al. 2004). The principle reason for conducting this study was to determine whether Cd could influence the content and composition of GAGs in the bone of rats.

Correspondence to: A. Galicka

MATERIALS AND METHODS

Our study was performed on three week-old female Wistar rats with the initial body weight of about 50 g. The rats were randomly assigned to the four experimental groups, each consisting of four animals. Three groups received an aqueous solution of $CdCl_2$ at the concentration of 1, 5 or 50 mg Cd/L, as their only drinking fluid, for a period of six months; control rats drank redistilled water (uncontaminated with Cd). At the end of the experiment, after overnight starvation, all animals were sacrificed under anaesthesia with Vetbutal (pentobarbital sodium and pentobarbital 5:1, 30 mg/kg b.w., i.p.). Femoral bones from the rats in each experimental group were assigned for GAG analysis.

Femoral samples free from adhering soft tissues were washed with 0.9% NaCl to remove bone marrow, and then defatted with chloroform/methanol (2:1, v/v) for 24 hr. The bone sample was then milled into a fine powder and the suspension was decalcified by dialysis against 3 × 1 L of 0.05 m*M* Tris-HCl buffer, pH 7.5 containing 0.5 *M* EDTA for 24 hr each. The decalcified bone matrix was dialyzed for 24 hr against 2 × 1 L of deionized water and then freeze-dried. Isolation of GAGs was carried out according to the methods of Van Amerongen et al. (1990) and Bańkowski et al. (1996). GAGs were released from bone PG core protein by extensive digestion with papain. The purified GAGs were dissolved in distilled water and determined quantitatively by use of a dye-binding assay (Frandale et al. 1982). Commercial preparations of highly purified GAGs (Sigma) were used as standards.

The purified GAGs were submitted to fractionation on a microcolumn with CF11 cellulose (0.3 x 6 cm) equilibrated with 1% cetylpyridinium chloride (CPC). Samples of 0.4 mL volume, containing 20-40 μg sulphated GAGs were applied to the column. Elution was performed using the following solvents: (A) 1% CPC; (B) 0.3 *M* NaCl; (C) 0.3 *M* $MgCl_2$; (D) 0.5% CPC in solution containing: n-propanol, metanol, acetic acid and water mixed in a volume ratio of 40 : 20 : 1.5 : 38.5; (E) 0.75 *M* $MgCl_2$ in 0.6% acetic acid; (F) 0.75 *M* $MgCl_2$; (G) 1.25 *M* $MgCl_2$. The solvents B, C, E and F contained 0.05% CPC. The concentration of GAGs in the column eluates was estimated according to Frandale et al. (1982). Untreated and treated GAGs with chondroitinase ABC were submitted to agarose gel electrophoresis (Gottlieb and Chavko 1987).

The results were submitted to statistical analysis using one-way analysis of variance (ANOVA) followed by the Kruskal-Wallis ranks test, accepting $p<0.05$ as significant.

RESULTS AND DISCUSSION

Toxic action of Cd in bone tissue is known, but the mechanism of this action is not fully understood yet (Kjellström 1992). Cd can affect both mineral and bone matrix content. It has been found that Cd can affect bone matrix by disturbing of collagen metabolism (Iguchi and Sano 1982). Whereas collagen fibers provide

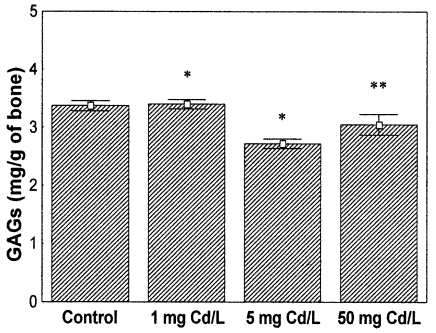


Figure 1. Effect of Cd on the total content of GAGs in the bone of control group and rats intoxicated with 1, 5 and 50 mg Cd/L. *P<0.05; **P<0.01.

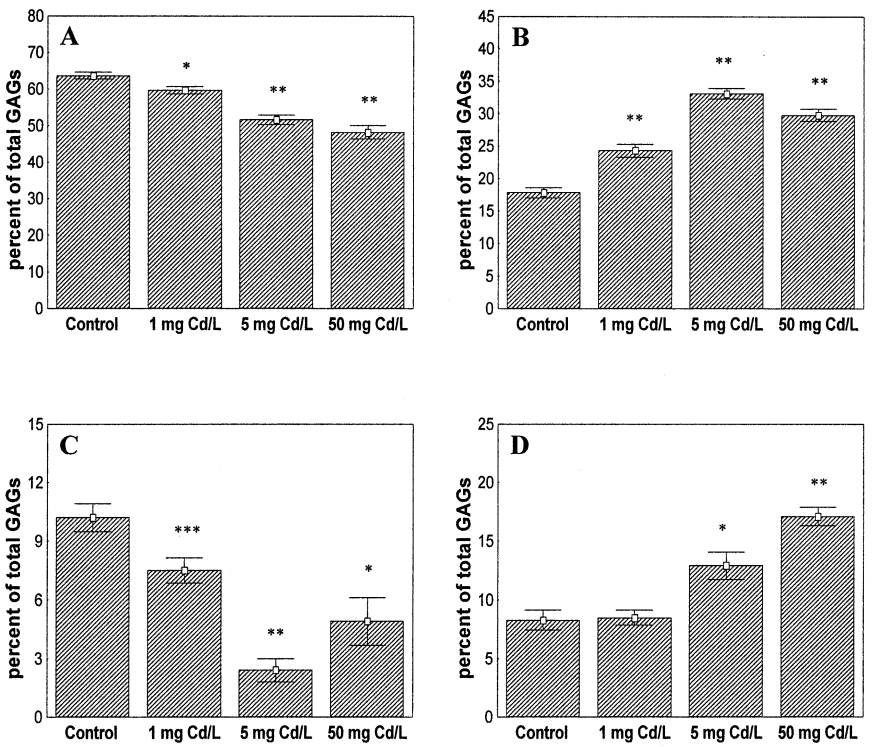
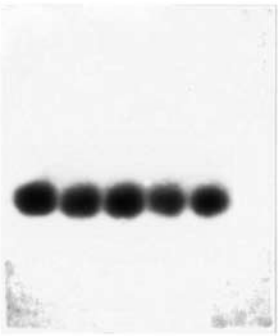


Figure 2. Effect of Cd on the C6S (A), C4S (B), KS (C), and DS (D) obtained during GAGs fractionation on the microcolumn with CF11 cellulose. *P<0.05; **P<0.01; ***P<0.001.

tensile strength, the GAGs and PGs form a polysaccharide gel that resists compressive forces on the matrix. The influence of Cd on content and



1 2 3 4 5 6

Figure 3. Agarose gel electrophoresis of bone GAGs of control (lane 2), and rats intoxicated with 1 (lane 3), 5 (lane 4) and 50 mg Cd/L (lane 5). Lane 1 shows CS marker, lane 6 - chondroitinase ABC treated GAGs.

composition of GAGs in bone of three week-old female rats was examined in the present work. As seen in figure 1, the total amount of GAGs in the bone was affected in rats exposed to 5 and 50 mg Cd/L. However, 5 mg Cd/L caused a slightly greater decrease in the total amount of GAGs (by about 19%) as compared to 50 mg Cd/L.

To determine the composition of GAGs, fractionation on a microcolumn with CF11 cellulose was performed. We identified three types of GAGs in the bone of control and intoxicated rats: CS, DS and KS. The main fraction, which consisted of about 80% of the total GAGs, was CS including C6S (Fig. 2A) and C4S (Fig. 2B). However, proportions of the two sulphated forms of CS were altered in bone of the intoxicated rats. The ratio of C4S to C6S increased 1.5 times in bone of rats exposed to 1 mg Cd/L, and 2.3 times in rats exposed to 5 and 50 mg Cd/L, as compared to the control values. These findings suggest that Cd can affect a pattern of GAGs sulphation.

KS and DS, as the minor fractions, accounted for about 10% each of the total amount of GAGs in the femur of control group (Fig. 2C,D). In the bone of intoxicated rats, the marked decrease in a percentage of KS to the total amount of GAGs was found (Fig. 2C). The most significant reduction, about 76%, was observed in the rats exposed to 5 mg Cd/L. In contrast, the percentage of DS to the total amount of GAGs was significantly increased in the bone of rats intoxicated with 5 and 50 mg Cd/L (1.5 and 2 times, respectively) (Fig. 2D).

The GAG fractions were verified by use of agarose gel electrophoresis. As can be seen in figure 3, the major fraction CS was only identified on the gel in the bone of both the control and intoxicated rats. The minor fractions, DS and KS, were not detectable on the gel. Chondroitinase ABC treatment of GAGs which leads to

removal of CS, HA, DS and retention of HS/H, KS, did not reveal any fraction of GAGs.

In summary, it was found that Cd affected the content and the GAG composition of PGs, as well as the sulphation pattern of GAGs in the bones of rats. Since PGs and their spatial arms, GAGs, play an important role in the regulation of collagen fibril assembly (Oegema et al. 1975) and mineralisation (Rees et al. 2002), any disturbances in them can in turn, negatively affect the bone strength. Therefore, we can conclude that decrease in bone mineralization and weakness of bone mechanical properties which we previously found in intoxicated rats, (Brzóska et al. 2004) can be at least partly attributed to alterations in GAGs content and their composition.

REFERENCES

Bańkowski E, Sobolewski K, Romanowicz L, Chyczewski L, Jaworski S (1996) Collagen and glycosaminoglycans of Wharton's jelly and their alterations in EPH-gestosis. Eur J Obstet Gynecol Reprod Biol 66:109-117

Beaty NB, Mello RJ (1987) Extracellular mammalian polysaccharides: glycosaminoglycans and proteoglycans. J Chromatogr 418:187-222

Brzóska MM, Majewska K, Moniuszko-Jakoniuk J (2004) Mineral status and mechanical properties of lumbar spine of female rats chronically exposed to various levels of cadmium. Bone 34:517-526

Brzóska MM, Moniuszko-Jakoniuk J, Jurczuk M, Gałażyn-Sidorczuk M, Rogalska J (2001) The effect of zinc supply on cadmium-induced changes in the tibia of rats. Food Chem Toxicol 39:729-734

Frandale RW, Sayers CA, Barrett AJ (1982) A direct spectrophotometric microassay for sulfated glycosaminoglycans in cartilage cultures. Connect Tissue Res 9:247-248

Gottlieb M, Chavko M (1987) Silver staining of native and denatured eucaryotic DNA in agarose gels. Anal Biochem 165:33-37

Hardingham TE, Fosang AJ (1992) Proteoglycans: many forms and many functions. FASEB J 6:861-870

Iguchi H, Sano S (1982) Effect of cadmium on the bone collagen metabolism of rat. Toxicol Appl Pharmacol 62:126-136

Järup L (2002) Cadmium overload and toxicity. Nephrol Dial Transplant 17(Suppl 2):35-9

Katsuta O, Hiratsuka H, Matsumoto J, Iwata H, Toyota N, Tsuchitani M, Umemura T, Marumo F (1994) Cadmium-induced osteomalacic and osteopetrotic lesions in ovariectomized rats. Toxicol Appl Pharmacol 126:58-68

Kjellström T (1992) Mechanism and epidemiology of bone effects of cadmium. IARC Sci Publ 118:301-10

Miyahara T, Miyakoshi M, Kozuka H (1980) Inhibitory effect of cadmium on the mineralization of embryonic chick femurs in tissue culture. Japan J Publ Health 27:323-328

Oegema TR Jr, Laidlaw J, Hascall VC, Dziewiatkowski D (1975) The effect of proteoglycans on the formation of fibrils from collagen solutions. Arch Biochem Biophys 70:698-709

Rees SG, Shellis P, Embery G (2002) Inhibition of hydroxyapatite crystal growth by bone proteoglycans and proteoglycan components. Bioch Bioph Res Comm 92:727-733

Van Amerongen JB, Lemmen AG, Tonino GJM (1990) Glycosaminoglycans in dental pulp. In: Inoki M, Kudo K, Olgart T (eds) Dynamic Aspects of Dental Pulp. Molecular Biology, Pharmacology and Pathophysiology, Chapman and Hal, London, pp 259-276

Wang H, Zhu G, Shi Y, Wenig S, Jin T, Kong Q, Nordberg GF (2003) Influence of environmental cadmium exposure on forearm bone density. J Bone Miner Res 18:553-560