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Effect of Cadmium Intoxication on Collagen and Elastin Content in Tissues of the Rat

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Cadmium produces a variety of pathological effects in various organs in experimental animals or in accidentally intoxicated humans. The mechanism of these phenonema has been the subject of numerous investigations (Friberg et al., 1974). Many of the observed toxic effects are thought to be the results of secondary deficiencies in such essential trace elements as zinc, copper and iron. Cadmium induced deficiency of these elements has been demonstrated by several workers (Ashby et al., 1980; Anke et al., 1971: Bunn and Matrone, 1966; Mills and Dalgarno, 1972; Sang-Hwan and Whanger, 1981). Metabolism of the fibrous components of connective tissue, i.e. collagen and elastin, requires the presence of some trace elements. Biosynthesis of collagen depends on the presence of iron (indispensable for hydroxylation of proline and lysine residues) as well as manganese (glycosylation of hydroxylysine residues) (Kivirikko and Risteli, 1976). Collagen maturation in extracellular space, based on cross-link formation, is catalysed by lysyl oxidase. Copper ions are necessary for the activity of this enzyme and they can be antagonized by zinc (Chvapil et al., 1973). It is also believed that elastin biosynthesis depends on the presence of some trace metals, especially copper (Narayan et al., 1978). Copper deficiency produces significant decrease in elastic tissue resistance, caused by diminished cross-link formation (Kadar, 1979; Waisman et al;, (1969). Experimental studies by Nagai et al. (1982) showed that cadmium treatment of rats produced an increase in the urinary excretion of collagen catabolities. It was also shown that cadmium intoxication influenced bone structure and foetal growth (Yoshiki et al., 1978; Webster, 1978). These two effects on connective tissue were probably accompanied by disturbances in collagen metabolism. Moreover, it is known that fungal collagenase activity was affected by cadmium (Rosenzweig and Pramor, 1986). In the present paper a decrease in collagen and elastin content, and impared extracellular maturation of the collagen fibres in some tissues of rats intoxicated with cadmium were described.

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MATERIALS AND METHODS

Male Wistar rats (180 10g body wt.) were divided into three groups (20 animals each). The two experimental groups were given aqueous solution of cadmium chloride, per os by gavage, in a daily dose of 0.01 and 0.03mM Cd/kg for 6 weeks. The control animals were given an equivalent amount of water. Rats were fed on commerical, pelleted chow and tap water. When the experiment was over the rats were killed by decapitation, and blood taken. Samples of liver, lungs, kidney, heart muscle, and skin from the abdominal region were taken at autopsy. Urine was collected in the last two days of the experiment with glass metabolic cages.

Collagen fractions were isolated from tissues with a procedure described by Grasedyck et al. (1974). In brief, tissue samples were homogenized, and neutral salt-soluble collagen was extracted with 0.45M sodium chloride at 4° C. Acid-soluble collagen was isolated with 0.5M acetic acid at 4° C, and insoluble collagen was removed with 5% trichloroacetic acid at 95° C. The amount of collagen in the extracts was measured at hydroxyproline content. Hydroxyproline was determined with the method of Drozdz et al. (1976) based on the reaction of Stegemann (1958). In this method, acid hydroxylysis was used to liberate hydroxyproline from proteins. Oxydation with chloramine T, and conversion to colour complex with p-dimethylaminobenzaldehyde were the subsequent steps of the assay, followed by the spectrometric measurement of the colour complex. The same method was applied for the assay of hydroxyproline in the blood serum. A short-lasting acid hydrolysis was used as initial step of determination of urinary hydroxyproline excretion according to Parekh and Jung (1970). Serum and urine hydroxylysine level was assayed according to Blumenkrantz and Asboe-Hansen (1973) as modified by Drozdz et al. (1978). After hydrolysis, free hydroxylysine was oxidated by sodium periodate to pyrroline-5-carboxylic acid, which during further oxidation formed a colour complex with p-dimethylaminobenzaldehyde. The absorption of the complex at 565 nm was measured. Elastin content in the tissues was measured as described by Robert et al. (1971). Total protein was determined according to Lowry et al. (1951) using bovine albumin as the standard solution. Statistical significance of the differences was analysed with the Student's "t" test.

RESULTS AND DISCUSSION

Treatment with cadmium did not affect the survival of the animals. Collagen content in the tissues of investigated groups of rats is summarized in Table 1. A decrease in total collagen content was found in all studied tissues except the lungs. This decrease was produced by loss of insoluble collagen and only a slight decrease in collagen soluble fractions. In the lungs, a slight increase in total collagen without significant changes in soluble/insoluble collagen ratio was shown. Elastin content was decreased in the liver, skin and the heart muscle. No changes were found in the lungs and kidneys (Table 2).

Table 1. Collagen content in the tissues of rats intoxicaed with cadmium*

	Total Collagen	Neutral Salt Soluble Collagen	Acid-Soluble Collagen	Insoluble Collagen	Soluble/Insoluble Collagen Ratio
SKIN					
Controls	2.113 ± 0.046	0.186 ± 0.011	0.219 ± 0.014	1.708 ± 0.054	0.237 ± 0.009
Cd 0.01mM/kg	1.765 ± 0.051[a]	0.165 ± 0.015[a]	0.195 ± 0.011[a]	1.405 ± 0.062[a]	0.256 ± 0.010
Cd 0.03mM/kg	1.479 ± 0.048[a]	0.171 ± 0.017[a]	0.190 ± 0.017[a]	1.118 ± 0.075[a]	0.323 ± 0.017[a]
HEART MUSCLE					
Controls	0.843 ± 0.085	0.078 ± 0.020	0.122 ± 0.037	0.643 ± 0.105	0.311 ± 0.024
Cd 0.01mM/kg	0.763 ± 0.064[a]	0.064 ± 0.025	0.095 ± 0.042[a]	0.604 ± 0.085	0.263 ± 0.035[a]
Cd 0.03mM/kg	0.730 ± 0.078[a]	0.056 ± 0.027[a]	0.087 ± 0.036[c]	0.587 ± 0.073	0.244 ± 0.027[a]
LIVER					
Controls	0.265 ± 0.036	0.025 ± 0.004	0.037 ± 0.005	0.201 ± 0.036	0.308 ± 0.015
Cd 0.01mM/kg	0.234 ± 0.025[a]	0.020 ± 0.006[a]	0.029 ± 0.006[a]	0.185 ± 0.027	0.265 ± 0.016[a]
Cd 0.03mM/kg	0.207 ± 0.028[a]	0.018 ± 0.007[a]	0.029 ± 0.008[a]	0.164 ± 0.030[b]	0.266 ± 0.020[a]
LUNGS					
Controls	0.978 ± 0.035	0.058 ± 0.008	0.345 ± 0.021	0.574 ± 0.038	0.704 ± 0.026
Cd 0.01mM/kg	1.031 ± 0.045[b]	0.067 ± 0.010[d]	0.372 ± 0.020[a]	0.592 ± 0.045	0.742 ± 0.020[e]
Cd 0.03mM/kg	0.035 ± 0.040[a]	0.065 ± 0.015	0.380 ± 0.025[a]	0.590 ± 0.060	0.754 ± 0.023[e]
KIDNEYS					
Controls	0.214 ± 0.018	0.020 ± 0.002	0.033 ± 0.003	0.138 ± 0.008	0.303 ± 0.018
Cd 0.01mM/kg	0.149 ± 0.020[a]	0.018 ± 0.003[e]	0.030 ± 0.006	0.101 ± 0.010	0.475 ± 0.027[a]
Cd 0.03mM/kg	0.152 ± 0.021[a]	0.017 ± 0.005[e]	0.030 ± 0.008	0.095 ± 0.018[a]	0.495 ± 0.030[a]

* Expressed as μmol of hydroxyproline lg wet tissue.
Statistical significance of differences from the corresponding controls of a - $P < 0.001$; b - $P < 0.002$; c - $P < 0.005$; d - $P < 0.01$; e - $P < 0.05$.

Table 2. Elastin content in the tissues of rats intoxicated with cadmium.

	Elastin [mg/g wet tissue]
SKIN	
Controls	0.308 ± 0.017
Cd 0.01mM/kg	0.345 ± 0.015[a]
Cd 0.03mM/kg	0.338 ± 0.013[a]
HEART MUSCLE	
Controls	0.276 ± 0.018
Cd 0.01mM/kg	0.250 ± 0.025[a]
Cd 0.03mM/kg	0.254 ± 0.019[a]
LIVER	
Controls	0.111 ± 0.010
Cd 0.01mM/kg	0.095 ± 0.011[a]
Cd 0.03mM/kg	0.081 ± 0.009[a]
LUNGS	
Controls	0.301 ± 0.018
Cd 0.01mM/kg	0.310 ± 0.025
Cd 0.03mM/kg	0.295 ± 0.038
KIDNEYS	
Controls	0.095 ± 0.010
Cd 0.01mM/kg	0.083 ± 0.013[b]
Cd 0.03mM/kg	0.080 ± 0.015[a]

Statistical significance of the differences from the corresponding controls a-$p < 0.001$; b - $p < 0.005$.

Serum levels of collagen metabolites and their urinary excretion were elevated in cadmium-treated animals as shown in Table 3. It was found that changes were generally dose-related, and alterations observed in rats treated with 0.3 mM Cd/kg were higher than those in animals receiving 0.01 mM Cd/kg.

The effects of cadmium on the animal body are complex, and the observed results are caused by direct and/or indirect metabolic and structural changes in various organs. Connective tissue has been found to be labile in the presence of numerous factors, including excess or deficiency of trace metals. Intracellular stages of collagen biosynthesis require iron and manganese. Deficiency in these trace elements leads to decreased formation of procollagen, a precursor protein of collagen. Extracellular formation of collagen fibres is initiated by conversion of procollagen to tropocollagen. The tropocollagen molecules spontaneously aggregate into fibres. Such fibres have little tensile strength, and cross-linking of collagen is a key phenomenon of extracellular maturation of fibres. The formation of strong, insoluble fibres depends on the development of

covalent crosslinks. This process is catalized by copper-dependent lysyl oxidase. Impared activity of the enzyme leads to decreased level of insoluble collagen. Similar cross-links formation occurs in elastin biosynthesis. The obtained results indicate that fibrous proteins of connective tissue are affected by cadmium intoxication. The mechanism of these changes is unclear. It is possible that cadmium-induced changes in copper and iron levels lead to decreased enzyme activity and diminished collagen biosynthesis and maturation. The observed changes are probably connected with disturbances in amorphous ground components (proteoglycans and structural glycoproteins) which played an important role in the formation and stabilization of collagen and elastin fibres. Systemic secondary mechanisms (e.g. hormonal changes influencing connective tissue) could also be involved in the development of the changes described.

Table 3. Serum and urine levels of collagen metabolites in rats intoxicated with cadmium.

	SERUM	
	[µmol/L]	[µmol/g of protein]
	Hydroxyproline	
Controls	112.9 ± 9.6	195 ± 0.16
Cd 0.01mM/kg	128.8 ± 11.3[a]	2.18 ± 0.21[a]
Cd 0.03mM/kg	134.0 ± 10.7	2.31 ± 0.22[a]
	Hydroxylysine	
Controls	37.1 ± 1.4	0.64 ± 0.05
Cd 0.01mM/kg	41.7 ± 3.4[a]	0.73 ± 0.08[a]
Cd 0.03mM/kg	48.5 ± 2.9[a]	0.88 ± 0.06[a]

	URINE	
	Hydroxyproline [µmol/24hr]	Hydroxylysine [µmol/24h]
Controls	1.28 ± 0.76	0.54 ± 0.28
Cd 0.01mM/kg	1.43 ± 0.74	0.81 ± 0.30[b]
Cd 0.03mM/kg	0.78 ± 0.77[c]	0.98 ± 0.29[a]

Statistical significance of differences from the corresponding controls: a - $p < 0.001$: b - $p < 0.01$: c - $p < 0.05$.

It is possible that collagen and elastin changes take part during the development of bone disturbances, Nagai et al. (1982) have shown an increased ratio of glucosyl-galactosyl-hydroxylysine to galactosyl-hydroxylysine in urine of cadmium-treated rats. This finding suggests that degradation of collagen occurs mainly in bone. Similar results were found in humans with the so-called Ouch-Ouch disease, occuring in Japan, and probably caused by chronic cadmium poisoning (Iguchi and Sano, 1974).

It is difficult to explain the reaction of connective tissue in the lungs resulting in an increased content of collagen. Organ susceptibility to cadmium, as well as types of collagen present in various organs could have moderated the extent of collagen changes in the investigated samples. Further studies to elucidate these phenomena are needed.

REFERENCES

Anke M, Henning A, Groppel B, Ludke H (1971) Der Einfluss des Kadmiums auf das Wachstum, die Fortpflanzungeslestung und den Eisen-, Zink-,und Kupferstoffwechsel. Arch Exp Vet Med 25:799-803

Ashby SL, King LJ, Parke DVW (1980) Effect of acute administration of cadmium on the disposition of copper, zinc, and iron in the rat. Environ Res 21:177-185

Blumenkrantz N, Asboe-Hansen G (1973) An improved method for the assay of hydroxylysine. Anal Biochem 56:10-15

Bunn CR, Matrone G (1966) In vivo interactions of cadmium, copper, zinc and iron in the mouse and rat. J Nutr 90:395-399

Chvapil M, Ryan JN, Elias SL, Peng YM (1973) Protective effect of zinc on carbon tetrachloride-induced liver injury in rats. Exp Mol Pathol 19:186-196.

Drozdz M, Kucharz EJ, Szyja J (1976) A colorimetric micromethod for determination of hydroxyproline in blood serum. Z Med Labortech 17:163-171

Drozdz M, Kucharz EJ, Szyja J, Kozarska-Samek E (1878) Studies on the optimal conditions of the colorimetric determination of total and free hydroxylysine in biological fluids and tissues. Z Med Labordiagn 19:294-305

Friberg L, Piscator M, Nordberg GF, Kjellstrom T (1974) Cadmium in the environment. CRC Press, Cleveland Ohio

Grasedyck K, Wulff U, Erl D, Lindner J (1974) Studies on collagen synthesis applying labelled proline. In: Connective tissues, biochemistry and pathophysiology. Fricke R, Hartman F (ed.). Springer Verlag, Berlin Heidelberg New York, p. 122-128.

Iguchi M, Sano H (1974) Effect of cadmium on proline metabolism and its relation to the urinary amino acid in "Ouch-Ouch disease". Jpn J Hyg 29:65-71

Itokawa Y, Abe T, Tabei R, Tanaka S (1974) Renal and skeletal lesions in experimental cadmium poisoning. Arch Environ Health 28:149-154 Kadar A (1979) The elastic fiber. Gustav Fisher Verlag, Jena, p. 49-53

Kivirikko KI, Risteli L (1976) Biosynthesis of collagen and its alterations in pathological states. Med Biol 54:159-186

Lowry OH, Resenbrough NJ, Farr Al, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275

Mills CF, Dalgarno AC (1972) Copper and zinc status of ewes and lambs receiving increased dietary concentrations of cadmium. Nature 239:171-173

Nagai Y, Sato M, Sasaki M (1982) Effect of cadmium administration upon urinary excretion of hydroxylysine and hydroxyproline in the rat. Tox Appl Pharm 63:188-193

Narayan AS, Page RC, Kuzan F, Cooper CG (1978) Elastin cross-linking in vitro. Biochem J 173:857-862

Parekh AL, Jung DH (1970) An improved method for determination of total hydroxyproline in urine. Biochem Med 4:446-456

Robert B, Szigeti M, Derouett JC, Robert L, Bousson H, Fabre MT (1971) Studies on the nature of "microfibrillar" component of elastic fibers. Eur J Biochem 21:507-516.

Rosenzweig WD, Pramer D (1986) Influence of cadmium, zinc, and lead on growth, trap formation, and collagenase activity of nematode-trapping fungi. Appl Environ Microbiol 40:694-696

Sang-Hwan OI, Whanger PD (1981) Effect of cadmium administration on absorption, retention, and excretion of zinc-65 administered to rats. Environ Res 26:130-135

Stegemann H (1958) Mikrobestimmung von Hydroxyprolin mit chloramin T und p-Dimethylaminobenzaldehyd. Z Physiol Chem 341:311-315

Waisman J, Carnes WH, Weissman N (1969) Some properties of the microfiblis of vascular elastic membranes in normal and copper-deficient swine. Am J Pathol 54:107-120.

Webster WS (1978) Cadmium-induced fetal growth retardation in the mouse. Arch Environ Health 33:36-42

Yoshiki S, Yanagisawa T, Kimura M, Otaki N, Suzuki M, Suda T (1978) Bone and kidney lesions in experimental cadmium intoxication. Arch Environ Health 30:559-562

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