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The effect of homocysteine thiolactone and its alpha methylated derivative on bone matrix in the mouse

Short Communication

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Summary. Homocysteine (HC) is a radiation protector but toxic to bone. Its derivative homocysteine thiolactone (HCTL) and the alpha-alkylated analogue (A-methyl-HCTL) was fed to mice for a period of six weeks in a daily dose of 50 mg/kg body weight. Parameters for bone matrix as collagen content, acid solubility of bone collagen, urinary bone collagen cross links (pyridinolines) and urinary acid glycosaminoglycans were determined. Urinary acid glycosaminoglycans were significantly reduced in the HCTL treated group but not in the alpha-methyl-homocysteine thiolactone (A-methyl-HCTL) group (controls: 45 ± 7 mg/mmol creatinine, homocysteine thiolactone 38 ± 5 mg/mmol creatinine, A-methyl HCTL 45 ± 6 mg/mmol creatinine).

No differences were found for the parameters of bone collagen between the groups. The potent radiation protecting methylated derivative therefore did not change bone matrix and should be a candidate for further toxicological studies.

Keywords: Amino acids – Homocysteine thiolactone – Alpha-homocysteine thiolactone – Bone – Glycosaminoglycans

Introduction

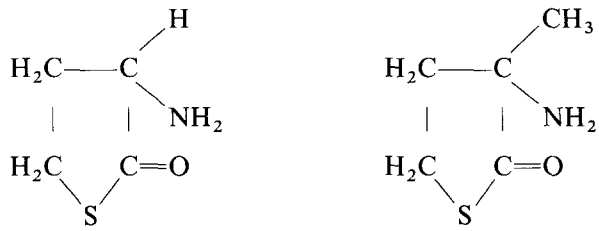
Homocysteine (HC) is a bioactive molecule and a metabolite of methionine metabolism. High homocysteine levels are found in homocystinuria, an inborn error of metabolism. One of the major defects and a consistent finding of this disorder are bone dysplasias (Cacciari, 1989). Bone dysplasias can be produced experimentally by oral administration of excess homocysteine (HC). The classical model is the experimental induction of tibial dyschondroplasia in chicken by high dietary homocysteine (Orth, 1991; Freedman, 1985).

A homocysteine derivative is of main interest to radiation chemists and therapists due to its radiation protection potential (Roberts, 1992).

HCTL effectively protects against ionizing irradiation but is not in use due to its tentative toxicity in high dosages used for this purpose (Mc Cully, 1989).

We modified homocysteine thiolactone by alpha methylation (Fig. 1) in order to reduce its toxicity and studied its effect on bone matrix (collagen and glycosaminoglycans) in the mouse system.

The formulae of both substrates, HCTL and α-MHCTL are given below:



Homocysteine thiolactone (HCTL)

α-Methylhomocysteine thiolactone (α-MHCTL)

Fig. 1.

Materials and methods

30 mice, white, female (Institute of Animal Breeding, Himberg, Austria) were used in the experiment. 10 animals were given homocysteine thiolactone (HCTL) (Sigma; DL-Homocysteine Thiolactone; Hydrochloride Crystalline HO 376; FW 153,6), 50 mg/kg body weight/day in tap water, 10 animals were used as controls and 10 animals were given orally alpha-methyl-homocysteine thiolactone (A-methyl-HCTL Hydrochloride Cristalline) 50 mg/kg body weight/day. The animals were treated for a period of six weeks. A-methyl-HCTL was synthesized by J. Häusler, Institute of Organic Chemistry, University of Vienna; the synthetic procedure will be published elsewhere.

At the end of the study period the animals were sacrificed by neck dislocation.

At autopsy tibial bone was taken for biochemical studies: A single urine portion was kept frozen along with the organs at −20°C.

Biochemical studies

Collagen content of bone

Tibial bone samples were weighed and hydrolyzed by incubation in 6 N HCl at 105°C for a period of 18 hrs. The hydrolyzate was spun down to remove undissolved material, filtered through Whatman filters (Puradisc 25. 6788-2502) and evaporated to dryness. The dried pellet was redissolved in 2 ml distilled water and hydroxyproline determined after the method of Woessner (1961).

Hydroxyproline values were expressed in μg hydroxyproline per mg bone.

Acid solubility of bone collagen

Acid solubility was evaluated by pepsin digestion of bone using 0,5 M acetic acid, 0,005 M EDTA, pepsin (Sigma 5 g P- 6887), 1 mg/100 mg bone tissue. 3 ml of this solution was added

to bone and incubated for 72 hrs at room temperature. To avoid microbial contamination sodium azide, 10 mg/100 ml was added.

After the incubation period the eluate was hydrolyzed under the conditions cited above. After evaporation the hydroxyproline determination after Woessner (1961) was applied and results expressed as μg hydroxyproline eluted per mg bone.

Urinary creatinine was determined by the Jaffe method.

Urinary pyridinoline cross links were determined by an ELISA technique (Collagen Crosslink™, Metra Biosystems Inc., USA Cat. No. 8701) and pyridinoline levels expressed as nmoles per mg creatinine.

Urinary acid glycosaminoglycans were determined after a standard method (Whitley, 1989) and expressed as mg/mmol creatinine.

Statistical analysis

Comparison of groups was performed by the Wilcoxon test using the SAS system, SAS Users Guide (1985).

A p less than 0. 05 was considered as statistical significance.

Results

Collagen content of bone

Table 1a presents the results of total collagen content of tibial bones.

Statistical comparison of the groups revealed no significant differences.

Table 1a

	Means	Standard deviation	Range
Controls	0.85	0.18	0.54–1.1
HC-TL	0.81	0.17	0.55–1.04
a-Met HC-TL	0.83	0.34	0.17–1.4

Total bone collagen content (expressed by μg Hydroxyproline/mg bone)

Acid solubility of bone collagen

Table 1b lists the result of the collagen acid salt extraction of collagen reflecting collagen cross linking. Statistically, no significant differences could be determined.

Table 1b

	Means	Standard deviation	Range
Controls	0.20	0.09	0.09–0.33
HC-TL	0.15	0.05	0.07–0.24
a-Met HC-TL	0.17	0.07	0.10–0.34

Elution of acid salt soluble collagen from bone (exp. μg Hydroxyproline/mg bone)

Determination of pyridinoline cross links

The evaluation of urinary pyridinoline cross links is shown in Table 2a as expressed by nmoles per mmole creatinine to rule out clearance influences.

Statistical analysis did not show significant differences between the groups.

Table 2a

	Means	Standard deviation	Range
Controls	305	80	156–380
HC-TL	337	54	278–450
a-Met HC-TL	308	40	240–368

Urinary pyridinoline crosslinks in nmols/mmol creatinine

Urinary acid glycosaminoglycans

Urinary acid glycosaminoglycans are shown in Table 2b and expressed in mg per mmoles creatinine. HCTL treated animals showed significantly lower excretion of acid glycosaminoglycans as compared to controls ($t = 1{,}9$ and $p\ 0{,}04$) or as compared to A-methyl HCTL ($t = 2{,}09$ $p = 0{,}03$).

Table 2b

	Means	Standard deviation	Range
Controls	45	7	31–53
HC-TL	38	5	33–50
a-Met HC-TL	45	6	37–57

Urinary glycosaminoglycanes in mg/mmole creatinine

Discussion

Radiation protection requires high doses of homocysteine thiolactone toxic to bone. Alpha-alkylation of homocysteine i.e. alpha-methylation, renders this compound unmetabolizable, which in turn enables the application of lower dosages. In a biological system we found furthermore higher radiation protective activity of A-methyl HCTL as compared to HCTL (unpublished results).

Effective doses for radiation protection can be evaluated as 50 mg per kg body weight per day. In this dosage neither homocysteine thiolactone nor alpha-methyl-homocysteine thiolactone led to changes in collagen content of bone, bone collagen cross links as expressed by acid salt solubility and urinary pyridinolines.

Bone changes could have been expected as homocysteine is known to inhibit collagen cross linking either by reaction with cross linking lysine aldehydes (Jackson, 1973), inhibition of lysyl oxidase (Lindberg, 1976) or decreased formation of polyfunctional cross links from hydroxylysinohydroxynorleucine (Siegel, 1975).

Mice treated with HCTL showed decreased urinary levels of acid glycosaminoglycans as compared to normal controls and mice given A-methyl HCTL. HCTL led to proteoglycan changes in cell cultures as reported by McCully (1989) most probably by acting on number or distribution of esterified sulfate groups. This explication would be compatible with our findings of reduced acid urinary glycosaminoglycans by HCTL. Toxicological differences between HCTL and its analogue, A-methyl-HCTL, can be explained by the modification of HCTL. A-methyl-HCTL is more lipophilic and therefore presents different pharmacokinetic and pharmacological properties. Another biochemical marker for bone damage, urinary pyridinolines, was unchanged by both compounds. Administration of this dosage would not lead to bone matrix and biochemical changes in the mammalian system but would be sufficient to exert its radiation protective effect.

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