ACONITATE HYDRATASE ACTIVITY AND CITRATE CONTENT OF HEART AND KIDNEY IN FLUORIDE AFFECTED COWS

by

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SUMMARY: Heart and kidney tissues from Holstein, Hereford and cross bred beef cows suffering from chronic fluoride toxicosis were analyzed for citric acid content and aconitate hydratase (citrate (isocitrate) hydro-lyase, E.C. 4.2.1.3) activity and the results were compared with those obtained with tissues from healthy cattle. Citric acid concentration was decreased 54 - 60% in kidney and heart of fluoride affected cattle. Aconitate hydratase activity in heart tissue showed an increase of about 25% in the heart tissue and a decrease of about 52% in the kidneys. The possible mode of action of these findings is discussed.

Introduction

Previous studies (1) indicated that Agropyron cristatum L. Gaerth (crested wheat grass) collected from an area high in atmospheric fluoride contained apparent trace amounts of fluoroacetate and fluorocitrate in addition to high levels of inorganic fluoride. Animals grazing on this vegetation exhibited chronic fluoride toxicosis. Although it is known that a significant increase of fluoride concentration in the bone ash of humans, bovines, laboratory rodents, and chickens resulted in reduction in citrate concentration (2-5), this finding was not confirmed in fluoride-affected cattle when citric acid was analyzed in bones on a dry fat-free basis (6). As some of the soft tissues from animals suffering severe osteofluorosis appeared to contain trace amounts of organically bound fluoride (7), it was deemed of interest to measure in these preliminary studies the citric acid content and aconitate hydratase activity in soft tissues of fluoride-affected cows as compared with the same parameters in soft tissues of healthy cows.

Materials and Methods

Soft tissues (hearts and kidneys) from 4 healthy cows served as controls. Tissues from twelve 5 to 12 1/2 year old cows from an area containing industrial fluoride-air pollution and exhibiting chronic fluoride toxicosis were obtained immediately after sacrificing the animals. Tissue extracts for citric acid determination were prepared as outlined

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by Buffa and Peters (8). The tissues were kept in an ice bath and extracts for citrate analyses were made within 2 hours after the death of animals. Citric acid was determined by the colorimetric method of Taylor (9) and the values are given in μg per gram wet tissue. Crude aconitate hydratase was extracted as follows: Twenty g of the fresh tissue was ground with 60 ml of cold citrate buffer (4×10^{-3}M, pH 4.7) in a Waring blender for 2 min., and the slurry was passed through four layers of cheesecloth and centrifuged at 20,000 x g for 20 min. The supernatant was suitably diluted with 0.1M phosphate buffer (pH 7.4) and used immediately. The spectrophotometric assay procedures were the same as described by Hsu and Miller (10). Protein was determined by the method of Lowry et al. (11). The specific activity was given as optical density Δ/min/mg of wet tissue.

Results

Citric acid concentration of the normal animals ranged from 21 to 49 micrograms per gram wet tissue (average 39.1) for hearts and 23 to 55 micrograms per gram (average 40.2) for kidneys. Concentrations of citric acid in tissues from fluoride-affected animals ranged from 9 to 30 micrograms per gram (average 15.7) for hearts and 8 to 34 micrograms (average 18.4) for kidneys. This represents a 60 to 54% decrease respectively as compared with control values (Table 1). The aconitate hydratase activity of the heart and kidney tissues exhibited a different pattern of change. Whereas the enzyme activity of the kidney tissue extract from the fluoride-affected animals decreased an average of 50%, that of the heart increased 25% over the controls (Table 2).

Table 1
Citric Acid Concentration of Soft Tissue

Animals	Number Tested	Citric Acid (μg/g wet Tissue)* Heart	Kidney
Control	4	39.1 ± 10.0	40.2 ± 12.0
Fluoride affected	12	15.7 ± 5.9	18.4 ± 5.6

*Sample mean and standard deviation.

Table 2
Aconitate Hydratase Activity of Soft Tissues

Animals	Number Tested	Aconitate Hydratase Activity* O.D. increase at 240 mμ/min/mg protein Heart	Kidney
Control	4	0.66 ± 0.126	0.462 ± 0.124
Fluoride affected	12	0.829 ± 0.183	0.22 ± 0.07

*Sample mean and standard deviation

Discussion

Shupe et al. (12) studied the metabolism of inorganic fluoride in dairy cattle and found that only insignificant amounts of fluoride were retained in soft tissues. The fluoride content of kidneys was somewhat higher than of other soft tissues, which mainly reflects the route of elimination of fluoride from the body (13). No gross or histologic changes due to fluoride were found in the soft tissues of cows ingesting as high as 109 ppm of fluoride. In the present study it was found that the citric acid concentration in the soft tissues of fluoride-affected animals was significantly decreased (54 to 60%), a finding similar to the pattern seen in bones of various animal species when exposed to high levels of fluoride (2,3,4,5). We feel that the similarity of pattern is not enough to prove the existence of identical mechanism, i.e., the competition between fluoride and citrate in bones. Long-term ingestion of excessive fluoride might reduce the availability of citrate in soft tissues, thus decreasing concentration, similarly to bones.

The elevated activity of aconitic hydratase in the heart and decreased activity in the kidneys seems controversial. Since fluorocitric acid is a strong inhibitor of aconitic hydratase both *in vitro* and *in vivo* (14), the decreased enzyme activity in kidney tissue might indicate its presence. This suggestion is supported by the failure of an earlier attempt to demonstrate its presence in the heart by gas chromatography, while it was detected in kidney of fluoride-affected animals (7). In our present study, the effects and detection of organically bound fluoride (fluorocitric acid) in soft tissues was not investigated. Therefore, it is difficult to interpret our findings related to the decreased and increased aconitate hydratase activities in various soft tissue of fluoride-affected cows. A further problem arises in the interpretation of decreased citrate levels in the soft tissues (hearts and kidneys) of fluoride-affected cows which should be higher than control values if fluorocitrate were present at least in the kidney. Since the kinetics of fluorocitrate formation in animals is unknown at present, further investigations should be done to elucidate the exact mechanism of aconitase-citrate-fluorocitrate and inorganic fluoride interrelationships in cattle affected by ingestion of excessive fluorides.

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