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Magnesium Deficiency and Cerium Promote Fibrogenesis in Rat Heart

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Cerium is a biologically active lanthanide and a major constituent of monazite. The observation that inhalation of particles of cerium causes pneumoconiosis had generated considerable interest in the toxicology of the element (Venugopal and Luckey 1978; Vocatura et al 1983). Cerium tartrate was found to produce cardiac injury and polycythaemia in small animals (Venugopal and Luckey 1978). More recently, tropical endomyocardial fibrosis (EMF), a restrictive cardiomyopathy, was postulated to be the cardiac expression of cerium toxicity in combination with magnesium deficiency (Valiathan et al 1989; Valiathan and Kartha 1990). The postulation was based upon the observation of elevated levels of cerium and depressed levels of magnesium in the cardiac tissue of patients with EMF (Valiathan et al 1989; Valiathan and Kartha 1990). Studies carried out in pursuance of the hypothesis showed that tissue levels of cerium are enhanced in magnesium deficiency (Eapen et al 1996) and that cerium and magnesium deficiency have a synergistic effect on cardiac metabolism (Gunther 1990; Shivakumar and Renuka Nair 1991). Importantly, recent observations on the mode of action of cerium at the molecular level suggested that the element may influence expression of matrix proteins like collagen in the heart and produce fibrosis (Prakash et al 1995; Shivakumar et al 1992). A sequel to these earlier investigations, the present study examined whether chronic ingestion of low doses of cerium would produce cardiac fibrosis in experimental animals. This communication presents evidence that cerium *per se* or in combination with magnesium deficiency produces subendocardial fibrosis and increase in interstitial cellularity and collagen content in rat heart. It also confirms the earlier observation from this laboratory that magnesium deficiency promotes accumulation of cerium in the cardiac tissue (Eapen et al 1996).

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MATERIALS AND METHODS

Magnesium- sufficient and -deficient diets, containing 0.0515% and 0.012% of magnesium respectively, were obtained from Ziegler Bros, USA. Feed composition based on the nutrient requirements for rats proposed by the American Institute of Nutrition is given in Table 1 (Anonymous 1977). Cerium chloride was from Sigma Chemical Company, USA.

Table 1. Composition of rat diet

Cellulose/glucose	50.0 g
Casein lactate	20.0 g
Corn starch	15.0 g
Cellulose	5.0 g
Fat: Corn Oil	5.0 g
American Institute of Nutrition Vitamin Mix	1.0 g
American Institute of Nutrition Mineral Mix	3.5 g
Dimethionine	0.3 g
Choline bitartrate	0.2 g

Sprague-Dawley rats of both sexes (M:F 1:1) weighing 55 ± 5g were randomly distributed into four experimental groups following a 2x2 factorial design. Animals in groups A and B were fed magnesium-sufficient diet while those in groups C and D were fed magnesium-deficient diet. Cerium chloride dissolved in drinking water to a concentration of 35 ppm was given to groups B and D while groups A and C were given double distilled water. Feed and water were provided *ad libitum.*

At thirteen months, animals were killed under ether anaesthesia and cardiac tissue was collected for elemental analysis and histology. Blood was collected at the start of the experiment and at the time of sacrifice and serum was separated and stored for elemental analysis. Levels of magnesium and calcium were determined by atomic absorption spectrophotometry (IL Model 551, USA) and cerium content of tissue was determined by inductively coupled plasma mass spectrophotometry.

Collagen content was calculated from the hydroxyproline content per unit tissue (Laurent et al 1978). Hydroxyproline in dry tissue was analysed following acid hydrolysis as described elsewhere (Mays et al 1991). Briefly, cardiac tissue was hydrolyzed in 6N HCl at 110°C for 16h, and the hydrolysate decolourized with activated charcoal. Hydroxyproline content was measured in the total hydrolysate following oxidation with chloramine-T. Molar amounts of pyrrole, the oxidation product of hydroxyproline, were determined after extracting the products into methylbenzene (Mays et al 1991).

RESULTS AND DISCUSSION

Rare earth elements like neodymium and cerium were used therapeutically for their anti-coagulant and anti-emetic properties until they were found to be toxic (Evans 1990). The use of cerium in industry and its possible role in the pathogenesis of pneumoconiosis and tropical EMF had drawn attention to the pathobiological events triggered by the element. Studies on acute toxicity following intravenous, intramuscular or intragastric administration of large doses of cerium have yielded useful insights into the biology of cerium (Evans 1990) but, surprisingly, the effect of chronic ingestion of low doses of the lanthanide has received little attention which is a lacuna that the present study has addressed.

Table 2. Serum levels of magnesium (Mg) and calcium (Ca)

Element	Group A		Group B		Group C		Group D	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Mg	2.09 ± 0.12 (5)	2.45 ± 0.16* (5)	2.15 ± 0.27 (8)	2.28 ± 0.21 (8)	2.22 ± 0.29 (9)	1.76 ± 0.31* (8)	2.26 ± 0.29 (8)	1.35 ± 0.18# (8)
Ca	10.50 ± 0.70 (5)	10.75 ± 0.33 (5)	10.55 ± 0.40 (8)	10.99 ± 1.25 (8)	10.61 ± 0.44 (7)	12.20 ± 0.68# (8)	11.18 ± 0.69 (8)	12.43 ± 0.67* (8)

Values are expressed as mean ± SD (mg/100ml serum)
Number in brackets represents number of animals used
Initial value vs. final value : * $p<0.01$; # $p<0.001$

The suspected causal role of cerium and magnesium deficiency in tropical EMF had led us to investigate their combined effects on the heart. This communication reports on the histological changes and changes in cardiac collagen content and elemental levels in serum and heart induced by cerium *per se,* magnesium deficiency and a combination of the two.

Changes in electrolyte levels included a lowering of serum magnesium levels in groups C & D, the drop being more marked in the latter (Table 2). Though not statistically significant, a small difference in cardiac tissue levels of magnesium was observed between groups A & C, but group D had a significantly lower cardiac magnesium level (Table 3). Serum calcium was significantly higher in groups C and D (Table 2) which is in agreement with the earlier reports that in rats, hypomagnesemia is associated with hypercalcemia (Elin et al 1971; Shils 1988). Calcium levels in the heart were also significantly higher in these two groups (Table 3). An important observation was the higher level of cerium in the cardiac tissue in group D (Table 3) compared to group B, confirming an earlier observation that

Table 3. Cardiac tissue levels of magnesium, calcium and cerium

Element	Group A	Group B	Group C	Group D
Magnesium (μg/mg tissue dry wt.)	0.898 ± 0.15 (6)	0.963 ± 0.07 (8)	0.827 ± 0.04 (9)	0.663 ± 0.04* (8)
Calcium (μg/mg tissue dry wt.)	0.574 ± 0.07 (6)	0.509 ± 0.05 (9)	0.924 ± 0.09# (8)	0.977 ± 0.05# (8)
Cerium (ng/mg tissue dry wt.	0.065 ± 0.078 (6)	0.86 ± 0.33 (9)	0.184 ± 0.15 (8)	2.895 ± 0.51# (8)

Values are expressed as mean ± SD
Number in brackets represents number of animals used
Compared to Group A : * $p<0.01$; # $p<0.001$

magnesium deficiency may promote accumulation of cerium in the cardiac tissue (Eapen et al 1996). It is noteworthy that in all these instances, the observed change is more marked in group D than in group B or C, suggesting that magnesium deficiency and cerium may act synergistically.

Histological changes in the heart in response to a magnesium deficient diet and cerium administration are shown in Figure 1 (A-D). As mentioned in the figure legend, the lesions included myocytolysis, contraction bands, myofibrillar lysis, interstitial fibrosis and foci of scarring, particularly in the subendocardial myocardium. One of the animals in group D had scarring around an intramyocardial artery. Three out of 9 animals in group B, 2 out of 9 in group C and 4 out of 8 animals in group D had such changes. Thus, the number of animals with histological lesions was more in group D than in group B or C. This is possibly due to enhanced levels of cerium in the tissue in magnesium deficiency, as shown in this study and elsewhere, or potentiation of the biological effects of cerium in magnesium deficiency.

Table 4. Collagen content of cardiac tissue

Tissue	Group A	Group B	Group C	Group D
Heart	7.31 ± 0.24 (6)	8.40 ± 0.30* (8)	8.64 ± 0.51* (8)	9.70 ± 0.67* (8)

Values are expressed as mean ± SD (mg/g dry tissue wt)
Number in brackets represents number of animals used
Compared to group A - * $p < 0.001$

Significantly, results presented in Table 4 show an increase in cardiac collagen content in groups B, C and D which is in agreement with the histological finding of fibrosis.

It was reported earlier from this laboratory that cerium stimulates collagen synthesis in cardiac fibroblasts (Shivakumar et al 1992). That the element induces subendocardial fibrosis and increases the collagen content of the heart, as reported in this communication, is thus consistent with its action at the molecular level. Taken together, these observations indicate that cerium and magnesium deficiency may promote fibrogenesis in rat heart.

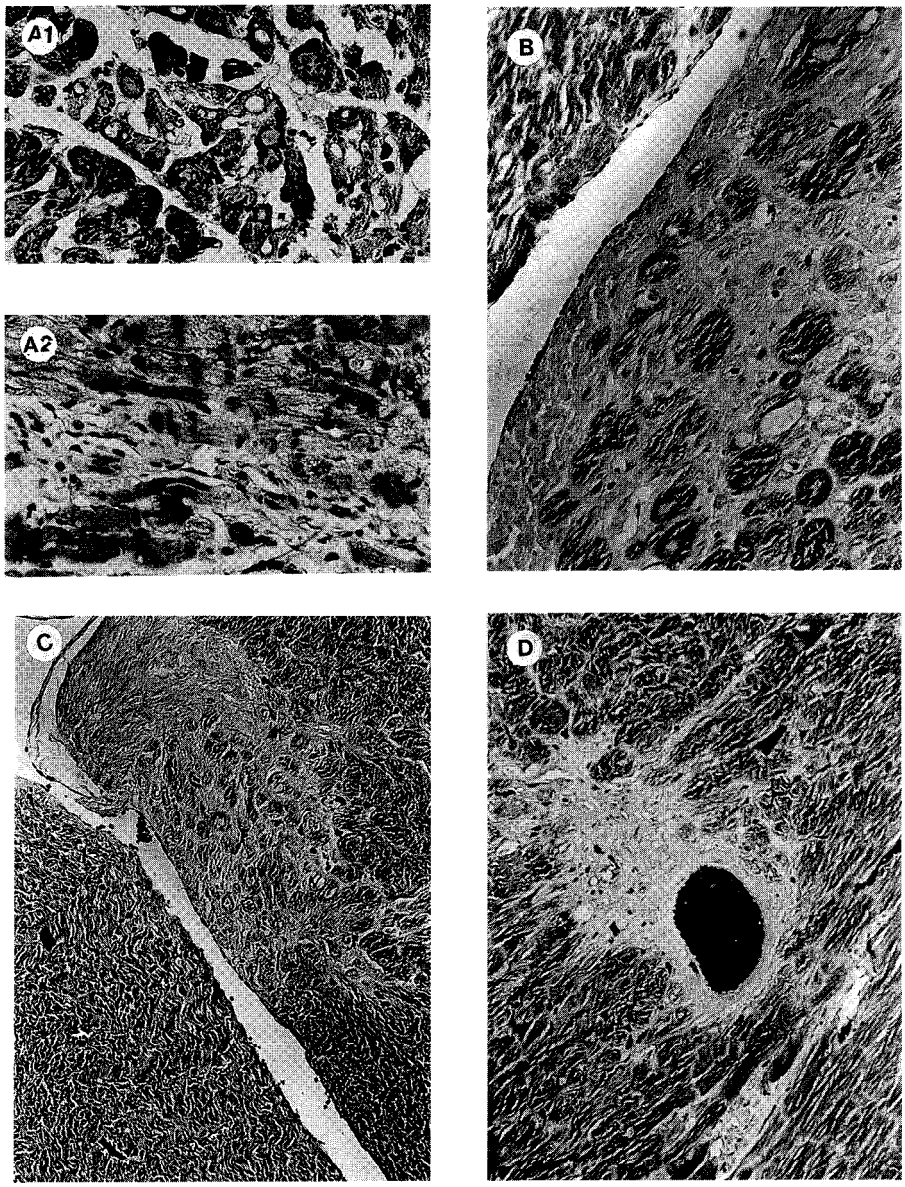

Figure 1. Photomicrographs of cardiac lesions in experimental animals

A. Myocytolysis (A1) and contraction bands and myofibrillar lysis (A2) are seen in the subendocardial region. Haematoxylin & Eosin x 240
B. Intermyofibre fibrosis in the myocardium. Some of the myofibres entrapped appear atrophic. Masson's trichrome x 240
C. Endocardial fibrosis with tongues of fibrous tissue creeping in between myofibres into subendocardial myocardium. Masson's trichrome x 120
D. An area of scarring around an intramyocardial artery. Masson's trichrome x 240.

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REFERENCES

Anonymous (1977) Report of American Institute of Nutrition ad hoc Committee on standards for nutritional studies. J Nutrition 107: 1340-1348

Eapen JT, Kartha CC, Rathinam K, Valiathan MS (1996) Levels of cerium in the tissues of rats fed a magnesium-restricted and cerium-adulterated diet. Bull Environ Contam Toxicol 56: 178-182

Elin RJ, Armstrong WD, Singer L (1971) Body fluid electrolyte composition of chronically magnesium-deficient and control rats. Am J Physiol 220:543-548

Evans CH (1990) Biochemistry of the lanthanides. Plenum Press; New York

Gunther T (1990) Magnesium deficiency generally enhances cytotoxicity. Magnesium Bull 12:61-64

Laurent GJ, Sparrow MP, Bates PC, Millword DJ (1978) Collagen content and turnover in cardiac and skeletal muscles of the adult fowl and the changes during stretch-induced growth. Biochem J 176:419-427

Mays PK, McAnulty RJ; Campa JS, Laurent GJ (1991) Age-related changes in collagen synthesis and degradation in rat tissues. Biochem J 276:307-313

Prakash KB, Sunita LDS, Shivakumar K, Rathinam K (1995) Cerium stimulates protein biosynthesis in rat heart *in vivo*. Biol Trace Element Res 50: 237-242

Shils ME (1988) Magnesium in health and disease. Ann Rev Nutr 8:429-460

Shivakumar K, Nair RR (1991) Cerium depresses protein synthesis in cultured cardiac myocytes and lung fibroblasts. Mol Cell Biochem 100:91-96

Shivakumar K, Nair RR, Valiathan MS (1992) Paradoxical effect of cerium on collagen synthesis in cardiac fibroblasts. J Mol Cell Cardiol 24:775-780

Valiathan MS, Kartha CC, Eapen JT, Dang HS, Sunta CM (1989) A geochemical basis for endomyocardial fibrosis. Cardiovasc Res 23:647-648
Valiathan MS, Kartha CC (1990) Endomyocardial fibrosis - the possible connection with myocardial levels of magnesium and cerium. Int J Cardiol 28:1-5
Venugopal B, Luckey TD (1978) Toxicity of Group III metals. In: Venugopal B and Luckey TD (eds) Metal toxicity in mammals. Plenum Press, New York, pp. 101-173
Vocatura G, Colombo F, Zanoni M, Rodi F, Sabbioni E, Pietra (1983) Human exposure to heavy metals; rare earth pneumoconiosis in occupational workers. Chest 83:780-783