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Effect of EDTA Chelation Therapy on Aortic Calcium in Rabbits on Atherogenic Diets: Quantitative and Histochemical Studies

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ABSTRACT: A group of rabbits were sub-divided into two subgroups, the first being placed on a standard laboratory diet while the second was placed on a high cholesterol containing atherogenic diet. The animals were either infused with EDTA, saline or nothing (in the controls) followed by sacrificing and the aortas were examined for calcium both microscopically, using calcium stains, and quantitatively. Results indicated significantly less calcium in the aortas of the EDTA infused animals when compared to both controls and saline infused animals.

Introduction

Chelating agents, particularly EDTA, have been available for years. This study continues a series of papers analyzing the effects of intravenous EDTA therapy. Past publications include research in mineral excretion patterns in EDTA infused patients[1], renal function[2,3], osteoporosis[4], various analyses of biochemical blood serum levels[5-9], vascu-

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lar occlusive disease[10,11] and atherosclerosis and related disorders[12,13] to mention only a few. Since the only way to assess what really happens to the artery following intravenous infusion of EDTA is at autopsy, it was decided for these studies to use an animal that develops atherosclerosis in a manner similar to humans, namely rabbits. As far as can be determined, this is the first attempt to chelate an experimental animal and to ascertain the effects of EDTA chelation therapy on the calcium content of the blood vessels, both quantitatively and ultrahistochemically. It attempts to answer the question: Is EDTA capable of removing dietarily induced plaque calcium under in vivo study conditions?

Materials and Methods

Thirty-six New Zealand albino (Oryctolagus cunniculus) rabbits each weighing 3 to 4 kg were individually caged and maintained on a diet of Purina Rabbit Chow (Ralston Purina Co., St. Louis, Mo.). These animals were placed on a therapeutic dose of tetracycline (Tetrachel-S, Rachelle Laboratories), 20 mg/day/rabbit, for a period of two weeks as a precaution against infection[14].

Twenty-four of the rabbits were placed on a cholesterol diet, which was prepared by adding a sufficient amount of a 10% solution of cholesterol in corn oil (Mazola) to the Rabbit Chow to give concentrations of 250 to 500 mg cholesterol per 100 gm diet. Additionally, 100,000 I.U. of Vitamin D3 (cholecalciferal, Sigma Chemical Co., St. Louis, Mo.) suspended in corn oil (Mazola) were given subcutaneously to these rabbits at three-day intervals every four weeks during the diet, for a total dosage of 1 million units each. Hass[15] has shown that this regimen consistently has produced a severe chronic calcified medial degeneration of the arterial system. The remaining twelve rabbits were maintained on an untreated Rabbit Chow diet to serve as controls.

Cholesterol fed animals remained on this atherogenic diet for a total of twenty-three weeks and then were placed on the untreated Rabbit Chow diet before chelation therapy infusions were begun. Four rabbits from this group remained on the atherogenic diet throughout the chelation process (Group D1 below). The rabbits were then divided into the following seven groups for infusion of disodium edetate (EDTA): A - control diet, no infusion; B - control diet, EDTA infusion; C - control diet, saline infusion; D1 - cholesterol diet throughout EDTA infusion; D2 - cholesterol/control diet, EDTA infusion; E - cholesterol/control diet, saline infusion; F - cholesterol/control diet, no infusion.

EDTA was administered via the marginal ear veins, alternating ears, utilizing a 30 cc syringe and a Harvard syringe pump. The dosage was 50 mg/kg of body weight, at a rate not greater than fifteen mg per minute[16]. Non-EDTA treated rabbits were infused with normal saline in the same manner, using equivalent volumes as determined from the EDTA dosage levels. Each rabbit was infused a total of twenty times on alternating days.

Histological studies on the rabbit aortas were done to ascertain the presence of atherosclerotic calcification. Dahl's method[17] utilizing alizarin red S and light green, SF yellow, SH, was employed to detect the presence of aortic calcium. Calcium salts, with the exception of calcium oxalate, are stained intense reddish-orange, with a pale green background counterstain, using this method.

The quantitation of tissue calcium levels in the rabbit aorta samples was accomplished using the direct microcomplexometric analysis of Mori[18]. This method employs complexometric titration of calcium with the disodium salt of 1,2-diaminocyclohexane-N,N,N′,N′,tetracetic acid (CDTA), in the presence of fluoresceinbismethylene-imidoacetic acid (calcein) indicator. All chemicals used were of analytical grade.

The statistical method employed to examine all quantitative results in this study was a test of significance for sample means. This method was chosen because of the relatively small sample sizes ($N<10$) and because the universal standard deviations were not known. All significant differences were recorded at the ($p<.05$) level.

Results

Groups A, B, and C: Control Diet

Six weeks after infusion, histological examination of the aortae from Group A rabbits revealed no lipid localization in the intima nor atheromatous plaques with calcification (Fig. 1a). The average aortic tissue calcium level (Table 1) was greater ($p<.05$) than found in those controls which were infused with EDTA (Group B).

In Group B and C rabbits, the aortae show no calcified atheromatous plaques (Fig. 1b, 1c) nor any evidence of lipid deposition upon histological examination. The average aortic tissue calcium (Table 1) was significantly less ($p<.05$) in the EDTA infused animals than the controls but the saline infused animals showed essentially the same (actually greater but not statistically significant) level of aortic calcium than the controls.

Groups D, E, and F: Cholesterol Diet

Each of these groups was maintained on the atherogenic diet until four weeks before infusion was started except subgroup D1, which remained on the cholesterol diet throughout infusion. Histological examination of the aortae of this group showed no medical calcification, but areas of lipid accumulation as noted in Figure 1d. The average aortic tissue calcium (Table 1) found in this EDTA infused group was significantly less ($p<.05$) than that found in experimental animals which were infused with saline (Group E) or not infused

FIGURE 1

Photomicrographs of aortae of rabbits on control (1a,1b,1c) and high colesterol atherosclerosis producing (1d,1e,1f) diets infused with either EDTA (1b,1d), saline (1c,1e) or non-infused controls (1a,1f).

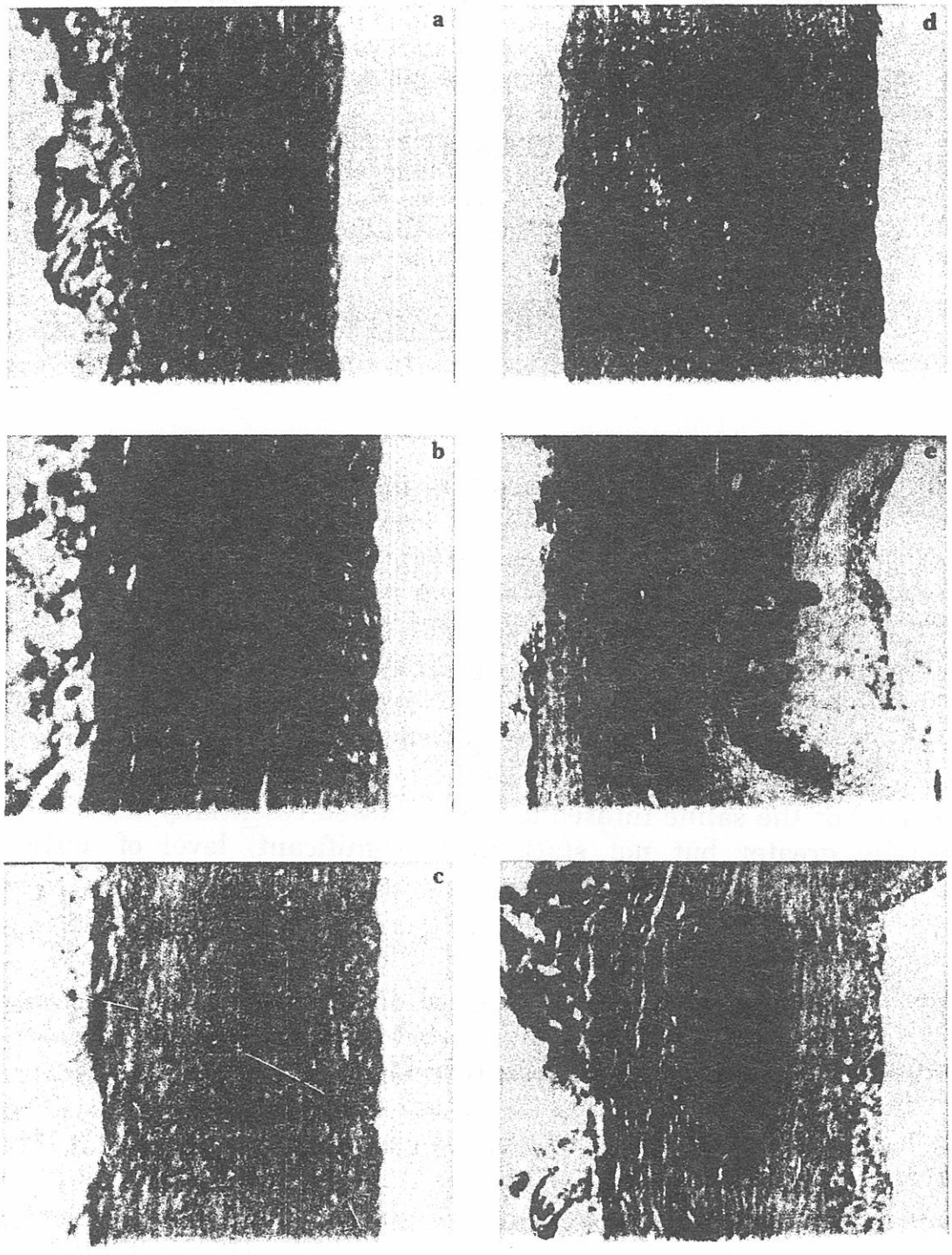

TABLE I

Aortic Tissue Calcium Levels

Group	Number of Animals	Diet	Infusion	Aortic Calcium-(microequiv-alents/gm)	N
A	3	Control	None	291	6
B	4	Control	EDTA	198*	8
C	4	Control	Saline	334	8
D_I	4	Cholesterol	EDTA	232*	8
D_{II}	4	Cholesterol/ Control	EDTA	351*	8
E	4	Cholesterol/ Control	Saline	635	8
F	3	Cholesterol/ Control	None	777	6

*Significant difference by t test ($p. < 05$)

(Group F). It was interesting to note that the D1 subgroup that was maintained on the cholesterol diet had significantly less ($p<.05$) calcium than those which were removed from the atherogenic diet before infusion (Group D2), which if applicable to humans, makes one question the emphasis placed on the low cholesterol diet in our patient counseling.

In Group E and F rabbits, considerable calcified plaque development and lipid deposition were shown by histological examination of the aortae (Fig. 1e, 1f). Aortic tissue calcium levels (Table 1) were significantly greater ($p<.05$) than calcium levels found in those experimental animals which were infused with EDTA (Group D), but not greater ($p<.05$) than the calcium found in the aortic tissue of experimental animals which were not infused (Group F).

Discussion

Historically, EDTA has been used to treat lead poisoning, digitalis intoxication[19] and certain collagen disorders[20]. Many physicians have

successfully used EDTA chelation therapy to treat atherosclerosis and related disorders[21]. Theoretically the chelate removes calcium deposits from the vessels, subsequently excreting bound calcium ions in the urine. This action may be mediated through the stimulation of the parathyroid glands[22,23,24], which may therefore contribute to the breakdown of atherosclerotic lesions by removal of metastatic calcium from the plaques[25].

From a simplistic point of view, the concept of chelation involves the use of a family of chemicals that are able to grasp metals with a claw-like action. The metal then becomes, in turn, incorporated into a multimembered ring structure and in doing so loses its physiological and toxic properties and is excreted from the body in this inert form. EDTA is a synthetic amino acid that forms strong soluble complexes with cationic minerals such as calcium, zinc, magnesium, lead and cadmium[26].

If EDTA is administered correctly with appropriate dosage, rate and concentration, it should produce no deleterious side effects. During the early stages of development, intravenous EDTA chelation was hindered by overdoses that resulted from not understanding the effective theapeutic range of this drug. The result of this incorrectly administered dosage was kidney, liver, and spleen damage, and even death[23,27,28]. The nature of this nephrotoxicity is not known but there is a definite association of increased vacuolar changes in the tubular epithelium of the kidney.

Recent studies have, however, found that safe levels of the chelate produce no renal damage and in fact even result in improved functioning[2,3]. Foreman[29] concluded that a dosage of 50 mg per kg per day would be safe for humans, and Meltzer[23] reported that 3 grams of disodium edetate per dose to be without danger of nephrotoxicity. If EDTA infusion results in improvement of these tissues as previously discussed, the only logical explanation is improved circulation.

Improvement in the circulation to a given tissue necessitates an "un-clogging of the pipe" that nourishes that tissue. The mechanism by which EDTA works has evolved away from the anti-calcification theory in part because the literature is focusing on reports of free-radical mechanisms as the basis of degenerative disease[30] and EDTA complexes metals that catalyze free radical auto-oxidation of the tissues. The other reason for this is that no one has shown the effect of EDTA on plaque calcium in an in vivo biological system. It is the hopes of the authors that the question of whether EDTA removes cal-

cium from atherosclerotic plaque in an in vivo situation has been answered.

Conclusion

There can be little doubt that disodium edetate produced a marked decrease in the plaques of induced atherosclerosis in these rabbits. Histological evidence indicated that calcified plaques were present in the animals which were fed the atherogenic diet and subsequently infused with saline or were not infused, but were absent from those that were infused with EDTA. Additionally, aortic tissue calcium was shown to be significantly reduced in both control and atherosclerotic animals which were infused with EDTA when compared to non-EDTA infused animals.

The precise anti-atherosclerotic mechanism is not known, but because calcium deposits in plaques are usually considered to be an end stage of atheroma formation, and pronounced calcifications are strongly associated with stenosis of the involved segments and ischemic myocardial lesions[31], it would seem plausible that removal of calcium could cause the plaque to become amorphous and either adsorbed or dissolved by the blood.

An alternate explanation might be that EDTA alters the interaction between lipids and components of the extracellular matrix so that the lipid material becomes more available for destruction. In this regard, it is possible that the altered availability of a metal ion might affect an enzymatic process or even a physical property, such as membrane permeability, with the resultant metabolic effects.

In summary, histological and histochemical examination of the aortae revealed, both qualitatively and quantitatively, that rabbits treated with disodium edetate had significantly less aortic calcium than those infused with normal saline or those which were not infused. It is hoped that this explains some of the results seen by the chelating physician with his patients.

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