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Rate of change of carotid intima-media thickness with magnesium administration in Abcc6$^{-/-}$ mice

Erine A. Kupetsky[1], **Fred Rincon**[2], and **Jouni Uitto**[3]

[1]Department of Dermatology, University of Pittsburgh Medical Center, Pittsburgh, PA 15213

[2]Department of Neurology and Neurosurgery, Thomas Jefferson University, Philadelphia, PA 19107

[3]Department of Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, PA 19107

Abstract

Pseudoxanthoma elasticum (PXE), caused by mutations in the ABCC6 gene, demonstrates progressive build-up of calcium phosphate and proteoglycans in the skin, eye, and arteries, and is associated to myocardial infarctions, stroke, blindness and elevated carotid intima-media thickness (CIMT). Although CIMT reduction with magnesium (Mg) has been documented in a mouse model for PXE (Abcc6$^{-/-}$), it is not clear if Mg is effective in humans with PXE to reduce CIMT. To examine this, we calculated the rate of change of CIMT (washout) in 15 month and 12-month-old Abcc6$^{-/-}$ mice fed standard rodent diet with or without Mg supplementation for 2 months. Using means in untreated 15-month and 12-month-old Abcc6$^{-/-}$ mice (145 μm and 120 μm, respectively), the rate of change was 8.3 μm/month. Using means in treated 15-month and 12-month-old Abcc6$^{-/-}$ mice (118 um and 104.6 um, respectively), the rate of change was 4.5 μm. Compared to normal progression of CIMT in humans without PXE, PXE has advanced atherosclerosis and possibly a higher CIMT rate of change. This experiment may portend, at least in PXE, the rationale for a one-year oral Mg CIMT clinical trial and may be useful for application in other progressive mineralizing disorders, like atherosclerosis.

Introduction

Pseudoxanthoma elasticum, PXE, caused by mutations in the *ABCC6* gene affects 1 100,000 to 1 in 25,000 individuals world-wide [1] and demonstrates progressive build-up of calcium phosphate and proteoglycans in the skin, eye, and arteries, and is consequently associated to myocardial infarctions, stroke, blindness and elevated carotid intima-media thickness (CIMT), a biomarker for cardiovascular morbidity and mortality [2–4]. Although CIMT reduction after pharmaceutical intervention has been documented in the literature [5–7], CIMT return to pathological baseline in progressive mineralizing disorders, especially in *Abcc6*$^{-/-}$

Address for Correspondence: Erine A. Kupetsky, D.O., M.Sc, Department of Dermatology, University of Pittsburgh Medical Center, 3601 Fifth Ave, 5th Floor, Pittsburgh, PA 15213, Tel: (412) 647-4956, Fax: (412) 647-4832, erine.rincon@gmail.edu.

Conflicts of Interest:
None

mice, has not been elucidated. Recently, in a mouse model of PXE, a magnesium (Mg)-enriched diet prevented mineralization of the vibrissae capsule, an early biomarker for PXE [8, 9], as well as significantly reduced CIMT in *Abcc6* $^{-/-}$ mice compared to controls [10].

This study aims to calculate the temporal relationship for CIMT to return to pathological baseline once Mg, a pharmaceutical intervention that significantly reduced CIMT in *Abcc6* $^{-/-}$ mice [10], is withdrawn. This data may be useful for application in other progressive mineralizing disorders, like atherosclerosis and may serve a greater role in understanding the length of time required for Mg to cause reversal change at the level of the carotid artery that is detectable and significant.

Methods

CIMT was measured in 15 month-old *Abcc6* $^{-/-}$ mice fed either standard rodent diet with or without Mg oxide supplementation (1.85grams/100grams food) for two months, followed by an additional two months of standard rodent diet. Carotid arteries were harvested from each panel and paraffin-embedded on numbered cassettes in a blinded fashion. Starting from the carotid bifurcation, the slides were H&E stained at 1000 μm, 3000 μm and 5000 μm) [10]. Slides were read using 40x magnification on a Nikon Eclipse TE 2000-U microscope (Mellville, NY, USA) and interpreted with calibrated Image-Pro Plus software (version 6.1.0.346 for Windows 2000/XP professional, Media Cybernetics, Inc., Bethesda, MD) [10]. Approval by Thomas Jefferson University's Institutional Animal Care and Use Committee prior to the start of these experiments was obtained. The mean of the CIMT measurements at 1000 μm, 3000 μm and 5000 μm mark distal to the bifurcation were used in the statistical analysis; each mouse's CIMT was then averaged to represent the final CIMT measurement from the treated or untreated *Abcc6* $^{-/-}$ group [10]. Values from both treated and untreated groups were analyzed for normal distribution using Hamilton's test. To determine the statistical significance between the treated and untreated groups of mice, a two-tailed Student's t-test was used [10]. To obtain the CIMT value in the 12 month-old mice, *Abcc6*$^{-/-}$ and *Abcc6*$^{+/+}$ mice were fed either standard rodent diet with or without the same amount of Mg oxide supplementation for two months and the same histology and statistical analysis were performed [10]. This was done in a previous experiment but a portion was reported here for fluency and comparison with the new data from the 15-month old mice. (Fig. 1a–d).

Results

As was anticipated, the baseline CIMT in all the control groups were increased with time (Fig 1a,b,e). (e) The CIMT in the untreated groups was 146 μm in 15-month old *Abcc6* $^{-/-}$ mice (mean, 145 μm, SD 7.12, $p<0.001$) compared to (b) 114 μm in 12 month-old *Abcc6* $^{-/-}$ mice (mean, 120 μm) [10] ; using means, this results in a calculated rate of change for the untreated group of 8.3 μm/month. In the Mg-treated group, (f), the actual CIMT of 119 μm (mean, 118 μm, SD 4.95, $p<0.011$), was similar to that of the (b) 12 month-old *Abcc6* $^{-/-}$ untreated group, (mean 120 μm, $p<0.001$). Also, the CIMT in the treated KO group was 90.5 μm (mean 104.6 μm) [10] (d); using means, this results in a calculated rate of change for the treated group of 4.5 μm/month (Fig. 1).

Discussion

While Mg reduces CIMT in a mouse model for PXE [10], the notion of whether Mg could be used in humans with PXE to reduce a known biomarker of morbidly and mortality, and for how long to be effective, is not so clear. This Mg washout experiment was required to help answer this quandary. According to the Cardiovascular Health Study and Northern Manhattan Study, normal CIMT ranges vary from 0.7 – 0.9 mm in 45 to 75 year-olds humans [11] and the progression of CIMT, depending on risk factors, can range from 0.01 ± 0.05 mm/year [12]. Although we used *Abcc6*$^{-/-}$ mice, humans with PXE have elevated CIMT compared to controls [4]. PXE, a progressive mineralizing disorder can also have advanced atherosclerosis, explaining the rate of change in CIMT in these mice. With Mg administration, the rate of change of CIMT per month was much less (4.8 um) versus without Mg (8.3 um), thus Mg may be useful for reducing CIMT in a rather rapid, short period of time if administered to PXE patients.

Conclusion

While some trials indicate that CIMT-reducing agents, like statins, may require five years to see any effect on CIMT in humans [6], this experiment may portend, at least in PXE, the rationale for a one-year oral Mg CIMT clinical trial. The time required for such CIMT changes to be evident and significant to reduce morbidity and mortality may be rapid in PXE.

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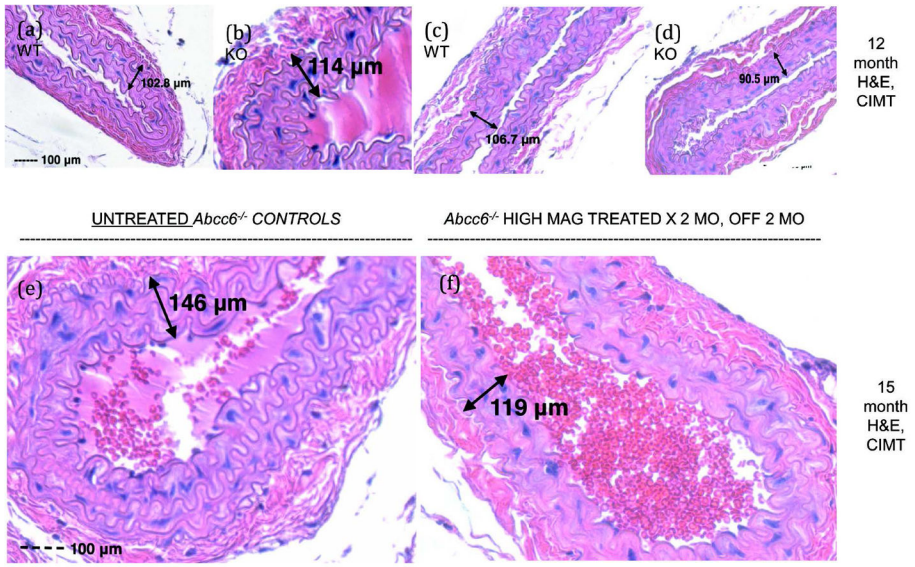

Figure 1.

Oral magnesium administration reduced CIMT in *Abcc6*$^{-/-}$ mice, and the rate of CIMT change is measurable in this mineralizing disorder. (a) 12 month-old carotid artery section from wild type (WT, *Abcc6*$^{+/+}$) controls (untreated) [10]; (b) 12 month-old carotid artery section from knockout (KO) controls (untreated) ($p<0.009$ compared to (a)) [10]; (c) 12 month-old carotid artery section from WT treated; (d) 12 month-old carotid artery section from KO treated with 2 months of oral magnesium ($p<0.02$ compared to (b)) [10]; (e) 15 month-old carotid artery section from a KO control mouse (untreated); (f) 15 month-old carotid artery section from KO mouse treated 2 months on, 2 months off with magnesium ($p<0.011$ compared to (e)). The mean value in (f), 118 µm, as well as the individual value shown, 119 µm, were similar to that of (b), the 12 month-old *Abcc6* $^{-/-}$ untreated group, mean 120 µm. The bar represents 100 µm.