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A Novel Chemical Solution to Demineralize Vascular Calcification in *Ex-Vivo* Amputated Limbs

Farres H[1], Yamani MH[2]*, Moualla S[2], Fuqua P[3] and Hakaim A[1]

[1]Departments of Vascular Surgery, Mayo Clinic Florida, Jacksonville, USA
[2]Cardiovascular Medicine, Mayo Clinic Florida, Jacksonville, USA
[3]Pharmacy, Mayo Clinic Florida, Jacksonville, USA

Abstract

The study was performed to evaluate the *ex-vivo* demineralizing efficacy of a novel chemical solution, combined L(+) lactic acid and D-gluconic acid (LAGA), in calcified peripheral arteries of 2 patients who underwent limb amputation. Angiography was obtained at baseline and after local installation of the 20 ml of LAGA solution in the occluded arteries. A significant dissolution of the calcification with restoration of patency of the occluded arteries was noted. LAGA solution is efficacious in demineralizing calcific peripheral arteries. Our study may pave the way for potential novel therapeutic strategies to treat patients with calcified peripheral arterial disease.

Keywords: Calcification; LAGA chemical solution; Amputation

Abbreviations: PAD: Peripheral Artery Disease; LAGA: Combined L(+) Lactic Acid and D-Gluconic Acid

Introduction

Peripheral arterial disease (PAD) is highly prevalent in the aging population, affecting 9 million Americans and 19% of those >70 years of age [1]. PAD is associated with a high 5-year risk of cardiovascular death, 25-30%, and an additional 20% risk of nonfatal major cardiovascular events [2]. The risk factors responsible for PAD are similar to those for coronary artery disease: advanced age, diabetes, hypertension, dyslipidemia and tobacco use [3]. The link between vascular calcification and advancing age has been well illustrated in the recent Horus mummy's study of ancient populations [4]. The economic and social burden of calcific PAD calls for the need of novel therapeutic interventions to alter the natural course of the disease process. In non-revascularizable patients with severe limb ischemia, 6-month major amputation rates have been reported to range from 10% to 40% [5]. Hence, the search for alternative effective therapeutic strategies that may be safe and natural is vital to attenuate the calcific disease process. The LAGA chemical solution is a combination of L(+) lactic acid (2-Hydroxypropionic Acid, $C_3H_6O_3$) and D-gluconic acid (2,3,4,5,6-Pentahydroxycaproic Acid, $C_6H_{12}O_7$) which are known to have demineralization properties and are naturally present in yogurt and honey, respectively [6-9]. We have recently shown that this combination is effective *in-vitro* in dissolving the calcification deposits in calcific aortic valves, mitral valves and coronary arteries [10]. The LAGA chemical solution is available in different concentrations and for the purpose of this study we used, the LAGA 15/5 (15% L(+) lactic acid and 5% D-gluconic acid based on our *in-vitro* study [10].

Our study is designed to test the hypothesis that LAGA chemical solution may be effective *ex-vivo* in demineralization of calcific PAD with the hope that it may serve as a potential therapeutic strategy in the future to improve circulation and may pave the way for future trials to reduce the need for limb amputation.

Methods

The study included 2 patients with severely calcific PAD who underwent limb amputation. Patient consent was obtained. These patients had angiography of the lower extremities as baseline, obtained preoperatively. The limbs specimens were retrieved, and the calcific diseased artery was isolated, cannulated proximally and clamped distally. A 20 ml of LAGA 15/5 was installed proximally and the artery was flushed with saline after a 45 minute period. Subsequently, an angiography was obtained and compared to the baseline study. The study was approved by the institutional review board of Mayo Clinic.

Results

Impact of LAGA 15/5

The LAGA 15/5 resulted in a complete restoration of the patency of the diseases arteries in both cases studied (Figures 1 and 2).

Discussion

Cardiovascular calcification is an age-linked active complex process where oxidized low-density lipoproteins, T-lymphocyte accumulation, autophagic cell death, osteoblast-like differentiation, and osteopontin producing macrophages have been shown to play major roles [11-13]. Recently, there has also been a paradigm shift in the understanding of pathogenesis of calcification from a passive atherosclerotic process to an actively regulated one involving activation of osteoblast cells and utilizing similar biochemical processes as in bone formation [14,15]. Vascular calcification has been emerged as a strong and independent risk factor for cardiovascular morbidity and mortality [16]. Since calcification is linked to aging and the latter is inevitable and irreversible, then a key question arises as to whether calcification should follow the same destiny as aging or could we alter the natural course of the disease by instituting early innovative therapeutic strategies. Our study introduces a novel LAGA chemical solution which has been shown to be effective in restoring patency of

***Corresponding author:** Yamani MH, Departments of Vascular Surgery, Cardiology, Mayo Clinic Florida, Davis 7E, 4500 San Pablo Rd, Jacksonville, FL 32224, USA, Tel: (904)953-7279; Fax: (904)953-2911; E-mail: yamani.mohamad@mayo.edu

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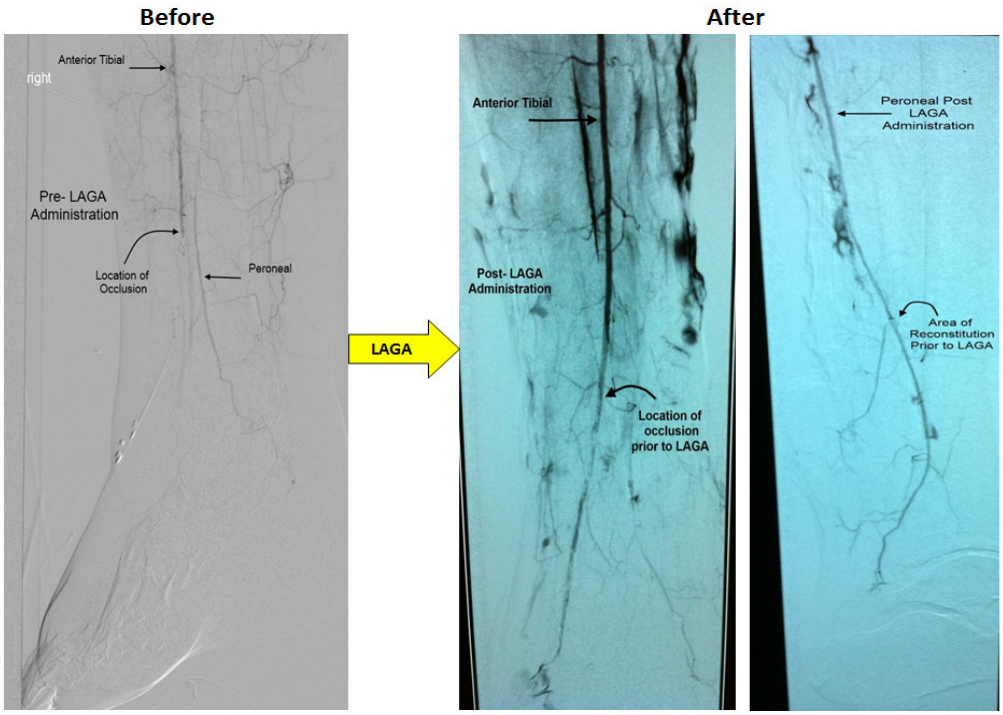

Figure 1: Both, anterior tibial and peroneal arteries are completely occluded at baseline and show evidence of patency of both arteries after LAGA administration.

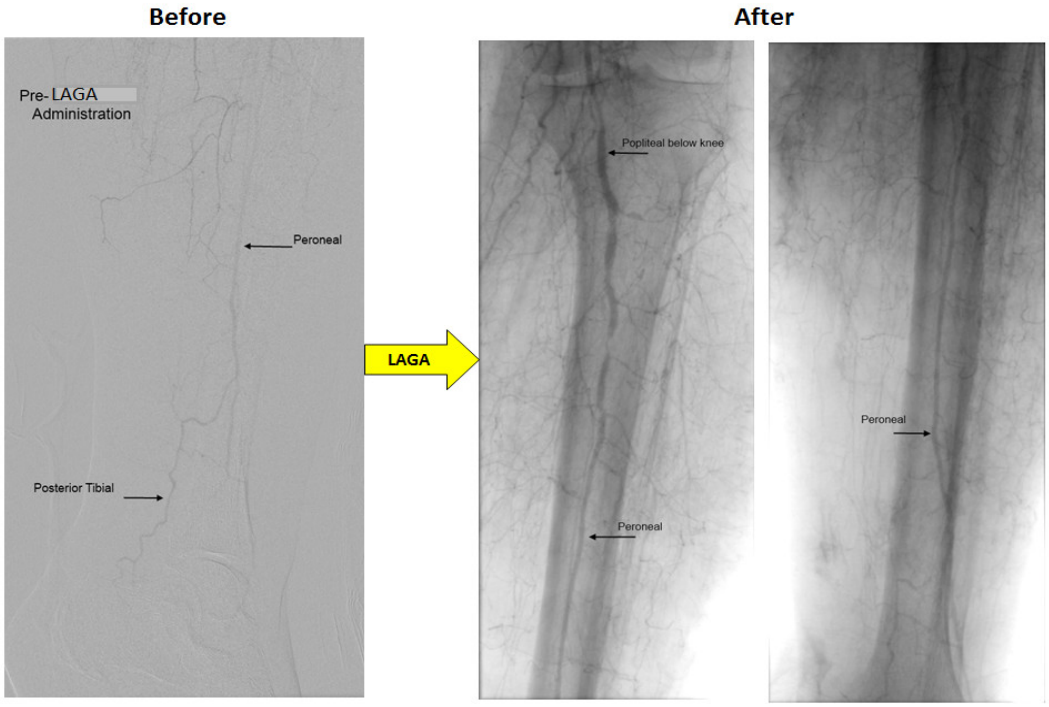

Figure 2: Severe occlusive disease of both, posterior tibial and peroneal arteries at baseline and show evidence of patency of both arteries after LAGA administration.

the disease arteries. We postulate that the chelating effect of LAGA with the transformation of calcium deposits to calcium lactate and calcium gluconate facilitates the demineralizing process. There has been a lot of enthusiasm in designing new therapeutic strategies to alleviate the vascular aging-degenerative process, but unfortunately, it was met with disappointing results. Cell-based regenerative therapies aiming at enhanced neovascularization and improved limb perfusion have been proposed as novel treatment strategies [17-19]. Recently,

the JUVENTAS trial has shown that repetitive intra-arterial infusion of autologous bone marrow mononuclear cells into the common femoral artery did not reduce major amputation rates in patients with severe, non revascularizable limb ischemia in comparison with placebo [20]. Ciccone et al. have evaluated the role of interventional therapy in diabetics with PAD and have made the observation that a rapid diagnosis and early prompt revascularization treatment are essential to improve quality of life and survival [21]. Our findings may pave the way for further clinical research to determine if LAGA chemical solution is effective in delaying the progression of vascular calcification and possibly reducing the rate of amputation.

Limitations

Our study has some limitations. We have a small sample size and further research is required to address this limitation. Our study introduces a novel concept but it does not address the mechanisms of calcification as it is beyond the scope of this study and hence, our study is mainly an observational one, though, with significant implications.

Conclusions

Our study shows that LAGA 15/5 is effective in restoring patency of the calcific PAD in these 2 cases. Further clinical research is required to verify our findings and determine if the use of LAGA 15/5 would have an impact on vascular outcome.

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