Biochemical and Biophysical Research Communications 432 (2013) 378–383

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

α-Lipoic acid attenuates coxsackievirus B3-induced ectopic calcification in heart, pancreas, and lung

Hyo Shin Kim [a,d,e], Hong-In Shin [b], Hyun-Sook Lim [c], Tae Yoon Lee [d], Kyunghee Lee [d,e,*], Daewon Jeong [d,e,*]

[a] Department of Clinical Pathology, Taekyeung College, Gyeungsan 712-719, Republic of Korea
[b] IHBR, Department of Oral Pathology, School of Dentistry, Kyungpook National University, Daegu 700-412, Republic of Korea
[c] Department of Public Health Administration, Hanyang Women's University, Seoul 133-793, Republic of Korea
[d] Department of Microbiology, Yeungnam University College of Medicine, Daegu 705-717, Republic of Korea
[e] Aging-Associated Vascular Disease Research Center, Yeungnam University College of Medicine, Daegu 705-717, Republic of Korea

ARTICLE INFO

Article history:
Received 11 January 2013
Available online 26 January 2013

Keywords:
Coxsackievirus B3
Ectopic calcification
Osteogenic signal
α-Lipoic acid

ABSTRACT

Ectopic mineralization of soft tissues is known to be a typical response to systemic imbalance of various metabolic factors as well as tissue injury, leading to severe clinical consequences. In this study, coxsackievirus B3 (CVB3) infection in mice resulted in significant tissue injury, especially in the heart and pancreas. Inflammatory damage and apoptotic cell death were observed in CVB3-infected heart and pancreas tissues. Along with tissue damage, substantial ectopic calcification was detected in CVB3-infected heart, pancreas, and lung tissues, as determined by von Kossa staining and calcium content quantification. In addition, CVB3 infection induced upregulation of osteogenic signals, including six genes (*BMP2*, *SPARC*, *Runx2*, osteopontin, collagen type I, and osterix) in the heart, three genes (*SPARC*, osteopontin, and collagen type I) in the pancreas, and two genes (*BMP2* and alkaline phosphatase) in the lung, as determined by quantitative real-time PCR analysis. Intriguingly, we showed that α-lipoic acid diminished CVB3-mediated inflammatory and apoptotic tissue damage, subsequently ameliorating ectopic calcification via the suppression of osteogenic signals. Collectively, our data provide evidence that ectopic calcification induced by CVB3 infection is implicated in the induction of osteogenic propensity, and α-lipoic acid may be a potential therapeutic agent to ameliorate pathologic calcification.

1. Introduction

Pathological ectopic mineralization has been reported to be responsible for serious clinical outcomes in humans [1]. Soft tissues such as the heart, kidney, stomach, lung, articular cartilage, and blood vessels are known to be susceptible to ectopic calcification [1,2]. Pathophysiological conditions that predispose soft tissues to abnormal calcification include microbial or viral infection, inflammation, metastatic cancer, aging, chronic kidney disease, diabetes, dyslipidemia, oxidant insults, hypercalcemia, and hyperphosphatemia [3–5]. Clinical classification of ectopic calcification generally encompasses both dystrophic and metastatic calcification. Dystrophic calcification is superimposed onto damaged tissues as a result of infection, necrotic cell death, and inflammation. For example, dystrophic calcification has been observed in tuberculosis lesions caused by pulmonary mycobacterial infection as well as in old infarcts [6]. Metastatic calcification is a cell-mediated, highly regulated process that represents calcium deposition onto undamaged tissues as a result of mineral imbalances in the circulating blood, leading to systemic extracellular matrix mineralization [5].

Final destined cells, considered to be terminal cells in specific tissues, can transdifferentiate into unwanted cells with entirely different properties distinct from intact cells. For instance, osteogenic transdifferentiation of cells within soft tissues results in localized and systemic calcification as well as irreversible dysfunction at the cellular and tissue levels. One of the most well known models of cell-mediated ectopic calcification is transdifferentiation of vascular smooth muscle cells into osteoblast-like cells, showing the formation of apoptotic bodies and membrane-bound matrix vesicles as well as the induction of osteogenic genes, including *BMP2*, *Runx2*, osterix, collagen type I, alkaline phosphatase, osteopontin, and osteocalcin [7–9]. To explore vascular calcification, murine knockout models of various genes encoding fibroblast growth factor 23, Klotho, matrix Gla protein, osteoprotegerin, fetuin A, apolipoprotein E, and low-density lipoprotein receptor as well as a nephrectomy model and stimulants, including vitamin D, nicotin, and warfarin, have been reported [10–13].

Despite these efforts, previous experimental animal models are not suitable for studying the pathological calcification process, and

* Corresponding authors. Address: Department of Microbiology, Yeungnam University College of Medicine, 317-1 Daemyung-Dong, Nam-Gu, Daegu 705-717, Republic of Korea. Fax: +82 53 653 6628.
E-mail addresses: kyungheelee@ynu.ac.kr (K. Lee), dwjeong@ynu.ac.kr (D. Jeong).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.01.061

it remains difficult to analyze the mechanism of ectopic calcification in other soft tissues except for aortic blood vessels. Our recent study reported that mice infected with coxsackievirus B3 (CVB3), human pathogenic picornavirus with a single-stranded RNA genome, show increased serum levels of inflammatory cytokines, including IL-1β, TNF-α, and the receptor activator of NF-κB ligand (RANKL), leading to substantial ectopic calcification in the heart, pancreas, and lung [14]. This model possesses time- and cost-saving advantages and can efficiently induce soft tissue calcification *in vivo*. Further, it was proven at the cellular level that RANKL is linked to *in vitro* cardiac fibroblast calcification by inorganic phosphate via induction of osteogenic signals, including *BMP2*, *SPARC*, *Runx2*, Fra-1, and NF-κB. In this study, we tried to analyze the multiple causative factors of ectopic calcification at the tissue level. We observed that CVB3-infected mice exhibited inflammatory and apoptotic tissue damage as well as osteogenic gene induction, specifically in the heart, pancreas, and lung. We further evaluated α-lipoic acid (1,2-dithiolane-3-pentanoic acid, ALA) as a potential therapeutic target for the prevention of ectopic calcification.

2. Materials and methods

2.1. Virus and mice

CVB3 (Nancy strain) was prepared in cultures of HeLa cells grown in DMEM (HyClone, Logan, UT, USA) supplemented with 10% FBS (HyClone) and antibiotics (Invitrogen, Carlsbad, CA, USA). Virus titer was measured in PFU/ml by plaque assay. Seven-week-old BALB/c mice were purchased from Central Lab Animal (Seoul, Korea) and maintained at the animal facility of Yeungnam University College of Medicine. All animal experiments were approved by the institutional review board of Yeungnam University Medical Center and were in compliance with the Guide for the Care and Use of Laboratory Animals.

2.2. Study design

Male mice at 7 weeks of age were injected intraperitoneally (i.p.) with CVB3 (2×10^4 PFU) in PBS or with PBS alone (control). ALA (10 mg/kg, Bukwang Pharm Corp., Seoul, Korea) was pre-injected 1 day before CVB3 injection. Then, ALA was injected the same time as virus administration as well as every other day thereafter. Mice were sacrificed on day 14 or at the indicated times after CVB3 infection for collection of soft tissues.

2.3. Histological analysis

Specimens fixed with 3.7% formaldehyde were embedded in paraffin and sectioned at a thickness of 4 μm. Paraffin-embedded sections of soft tissues were deparaffinized and subjected to hematoxylin and eosin (HE) staining for histological analysis or von Kossa staining for analysis of calcium deposition. Images were scanned with an Aperio ScanScope Model T3 and were analyzed with ImageScope software (Aperio Technologies, Vista, CA, USA).

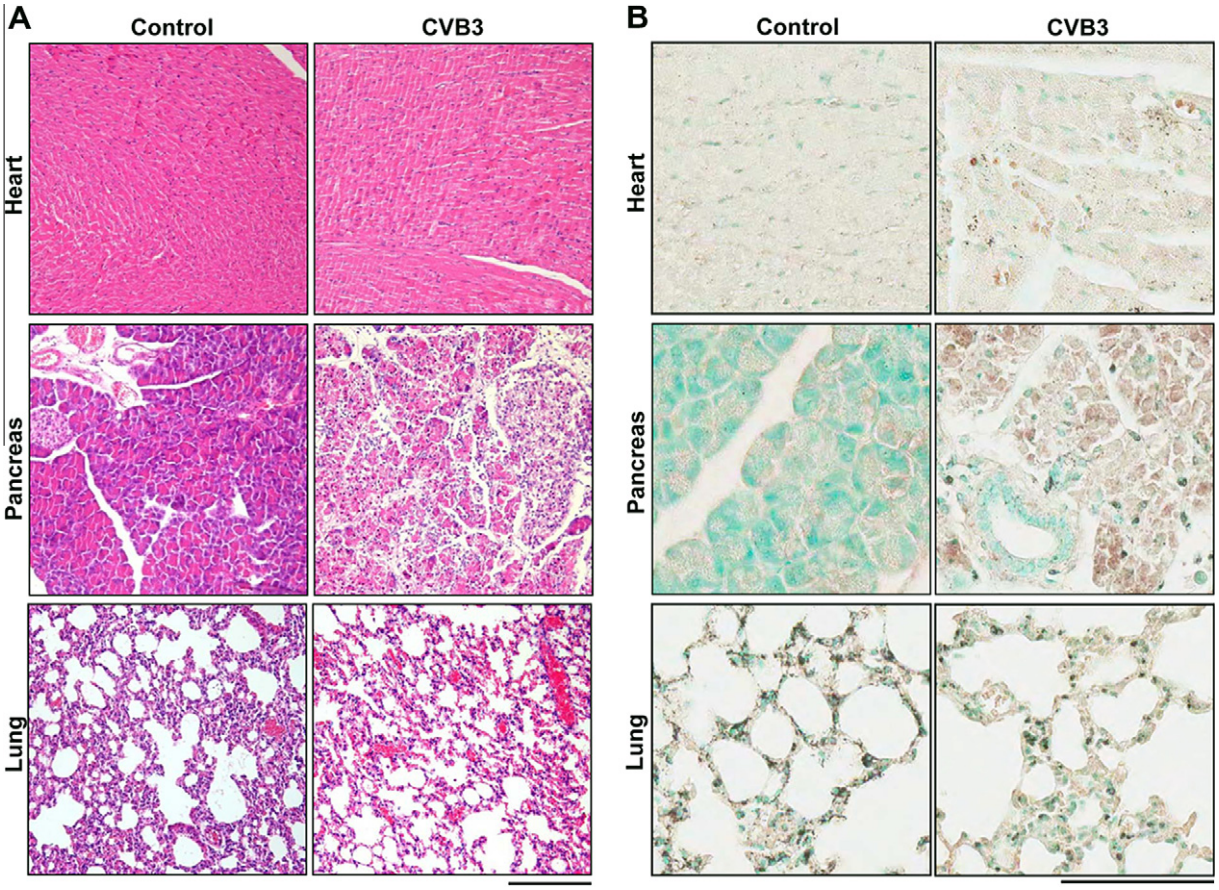

Fig. 1. Histological analysis and TUNEL assay of tissues from CVB3-infected mice. (A) Tissue sections from control ($n = 7$) and virus-infected ($n = 7$) mice were stained with HE at 3 days after CVB3 infection. Representative images are shown. Scale bar, 100 μm. (B) Assessment of apoptosis in heart, pancreas, and lung tissues from control ($n = 7$) and CVB3-infected mice ($n = 7$) at 14 days post-injection. Abundant brown staining of CVB3-infected heart and pancreas is shown. Representative images are shown. Scale bar, 100 μm.