

CHAPTER 1

INTRODUCTION

1.1. Background

Vascular calcification is the pathological condition that causes the deposition of calcium phosphate minerals in soft tissue (Schantl et al., 2020). The unstable elastic performance caused by vascular calcification might disturb windkessel physiology function and promote heart failure. In developed countries, people in elderly adults produce vascular calcification and degeneration of the aortic cusps as their aortic valve stenosis causes (Pohle et al., 2001). Vascular calcification is also included in the mineral metabolism disease type that promotes by several conditions like aging, renal function disease, and type II diabetes Mellitus condition (Towler, D.A., 2008).

In chronic kidney disease (CKD) patients, vascular calcification (VC) is one of the common complications that can be a predictor of vascular mortality due to the type of histoanatomic and the extent of VC (Mizobuchi et al., 2009). Moreover, VC is also a characteristic of some rare diseases, as well as pseudoxanthoma elasticum and generalized arterial calcification of infancy (Bartstra et al., 2019). Currently, no effective pharmacological is administered to prevent or treat vascular calcification conditions (Schantl et al., 2018). There is some drug that is under clinical investigation (like bisphosphonates, vitamin K, etc.) that act by stabilizing the imbalance calcification promoters or drug that act as inhibitors of vascular calcification. However, these drugs still do not prove efficacy under cardiovascular disease conditions (Schafer et al., 2003). Several variables that include in controlling the formation and

stabilization of minerals complex are Fetuin-A (Jahnen-Dechent et al., 1997), matrix GLA protein (Fleisch, H. & Bisaz, S., 1962), and Pyrophosphate (Heiss, A et al., 2008).

In normal conditions, the case of a high level of plasma phosphate or calcium, supported by a low concentration of calcification inhibitors, might be buffered by acidic serum proteins, especially fetuin-A, fetuin-A takes a role as a CPPs producer (Herrmann, M. et al., 2012). Nanoparticles from the primary CPPs (CPPI) that have calcium and phosphate spherical complexes with fetuin-A prevent deposition of calcium and phosphate (Pasch, A., et al., 2012). In the VC disease condition, there is the condition where delaying calcification and the balance between CPPI formation and absorption is disturbed. This condition results in the larger CPP, crystalline and spindle shape CPP is found in the vascular area (Köppert, S. et al., 2018). In the human body, primary and secondary CPP are cleaned by liver sinusoidal endothelial cells and liver Kupffer cells. There is a scavenger receptor called scavenger receptor A, a significant endocytosis receptor for secondary CPPs (CPPIIs) but not CPPI. This fact strengthens that there are different clearance pathways between primary and secondary CPPs (Chen, W. et al., 2019). CPPs are thought to play a role in the pathophysiology of VC in CKD (Miura., Y. et al., 2018). Serum CPP levels rise when renal function declines and are linked to coronary artery calcification, arterial stiffness, serum phosphate, inflammation, and procalcific factors (Hamano, T. et al., 2010). Furthermore, the Rh of CPPIIs was linked to VC in patients with severe CKD (Chen, W. et al., 2019). CPPIIs can cause vascular smooth muscle cells (VSMC) calcification in vitro might lead to a valid therapeutic target in vascular calcification research (Schantl et al., 2020).

MC3T3-E1 cell is one of the murine calvaria pre-osteoblast cell lines widely used in bone research. It has been cited more than 4000 times since it was first discovered in 1981 (Kodama, H., et al., 1981). MC3T3-E1 cells have several subclones divided into ‘mineralizing’ and ‘non-mineralizing’ characterization based on their expression of several hallmark osteoblastic genes, alkaline phosphate activity, and ability to store the mineralized matrix in vitro and/or in vivo (Wang, D., et al., 1999). MC3T3-E1 cells use to examine the signaling by way of the parathyroid hormone receptor (PTH1R) in bone metabolism (Moriya, S., et al., 2015). After the initial plating of MC3T3-E1 cells, cells get into a proliferative phase characterized by the synthesis of collagenous ECM and show terminal differentiation and minimal expression. Once matrix synthesis starts, it activates osteoblast marker genes in temporal order; alkaline phosphatase (ALP) and the parathyroid hormone (PTH)/parathyroid hormone-related protein (PTHrP) receptor at early times are induced (Stein., G.S., et al., 1990). Collagen matrix synthesis is compulsory for induction of marker differentiation, and a cofactor for collagen synthesis like ascorbic acid (AA) is required for osteoblast differentiation in vitro and in vivo (Mahmoodian F., et al., 1996).

Our recent laboratory experiment resulted in the CPP II effect on MC3T3-E1 cells in vitro and observed that calcification is found during the treatment of the cells. Furthermore, we try to observe their calcification phase to understand the exact time of early-stage calcification under CPPIIs treatment in MC3T3-E1 cells.

1.2. Research Question

Does the CPPIIs treatment affect the calcification on MC3T3-E1 cell culture?

1.3. Purpose

To investigate the calcification expression of MC3T3-E1 cell under CPPIIs treatment

1.4. Research Benefit

The in vitro study of MC3T3-E1 cell under the CPPIIs treatment might show the best time for an early calcification stage and vital for further research of drug candidates.