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Review

The role of carbonic anhydrase in the pathogenesis of vascular calcification in humans

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ABSTRACT

Carbonic anhydrases are a group of isoenzymes that catalyze the reversible conversion of carbon dioxide into bicarbonate. They participate in a constellation of physiological processes in humans, including respiration, bone metabolism, and the formation of body fluids, including urine, bile, pancreatic juice, gastric secretion, saliva, aqueous humor, cerebrospinal fluid, and sweat. In addition, carbonic anhydrase may provide carbon dioxide/bicarbonate to carboxylation reactions that incorporate carbon dioxide to substrates. Several isoforms of carbonic anhydrase have been identified in humans, but their precise physiological role and the consequences of their dysfunction are mostly unknown. Carbonic anhydrase isoenzymes are involved in calcification processes in a number of biological systems, including the formation of calcareous spicules from sponges, the formation of shell in some animals, and the precipitation of calcium salts induced by several microorganisms, particularly urease-producing bacteria. In human tissues, carbonic anhydrase is implicated in calcification processes either directly by facilitating calcium carbonate deposition which in turn serves to facilitate calcium phosphate mineralization, or indirectly via its action upon γ-glutamyl-carboxylase, a carboxylase that enables the biological activation of proteins involved in calcification, such as matrix Gla protein, bone Gla protein, and Gla-rich protein. Carbonic anhydrase is implicated in calcification of human tissues, including bone and soft-tissue calcification in rheumatological disorders such as ankylosing spondylitis and dermatomyositis. Carbonic anhydrase may be also involved in bile and kidney stone formation and carcinoma-associated microcalcifications. The aim of this review is to evaluate the possible association between carbonic anhydrase isoenzymes and vascular calcification in humans.

1. Introduction

Carbonic anhydrases are a group of zinc-containing isoenzymes that catalyze the reversible conversion between carbon dioxide (CO_2) and bicarbonate (HCO_3^-) (Fig. 1). In humans, they participate in a variety of biological processes, including the exchange of carbon dioxide in tissues and alveoli that allows respiration and the formation of a number of body fluids, such as pancreatic juice, gastric secretion, saliva, aqueous humor, cerebrospinal fluid, and sweat. They also contribute to the final composition of urine and bile and are likely involved in the generation of kidney and bile stones. Carbonic anhydrase is a pharmacological target and inhibitors of this enzyme have been used to treat a number of disorders including glaucoma [1], obstructive sleep apnea [2], altitude sickness [3], epilepsy [4], and metabolic alkalosis [5]. Carbonic anhydrase inhibition may play a role in the effect of topiramate inducing weight loss and glycemic control in type 2 diabetes, although the pathogenesis of these changes is uncertain [6]. In addition, carbonic anhydrase isoenzymes participate in metabolic reactions that are involved in calcification processes, such as the formation of calcium carbonate and the reaction catalyzed by γ-glutamyl carboxylase. Therefore, carbonic anhydrase isoenzymes may be implicated both in bone metabolism and in the formation of ectopic calcifications in soft tissues. However, the potential role of carbonic anhydrase in vascular calcification has been barely investigated in humans.

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2. Vascular calcification

The arterial wall in humans is composed of three layers: the inner layer or intima, the middle layer or media, and the outer layer or adventitia. The intima consists of a single layer of endothelial cells that rest on a basement membrane. A layer of elastic fibers, the elastica interna, encircles the endothelial basement membrane and is surrounded by smooth muscle cells and elastic fibers embedded on an extracellular matrix rich in collagen and proteoglycans. The elastica externa is a second stratum of elastic fibers that separates the media from the adventitia, which is composed of fibroblasts, elastic fibers, extracellular matrix, nerves, and small arteries that supply blood to the arterial wall [7]. The endothelial basement membrane contains type IV and type V collagen while collagen type I and type III predominate in the media and adventitia [8].

The wall of human arteries becomes frequently calcified with aging and in association with disorders such as pseudoxanthoma elasticum, diabetes mellitus, and chronic kidney disease.

Two types of arterial calcification have been described in humans. Calcification involving the intima layer is characteristic of the atherosclerosis lesion whereas calcification of the media occurs typically in patients with diabetes mellitus and chronic kidney disease, being termed Mönckeberg's medial calcinosis. Arterial calcification in patients with diabetes mellitus and chronic kidney disease may be extensive, as they manifest both types of calcification [9,10].

In humans, the earliest phase of the atherosclerosis process is the fatty streak, which consists of subendothelial accumulation of lipid laden macrophages (foam cells) and lipid deposits, leading to pathological intimal thickening. The next stage is the formation of the fibroatheroma plaque, which consists of a necrotic core thought to arise from macrophages and T lymphocytes infiltration producing cellular necrosis. A layer of fibrous tissue referred to as the fibrous cap enfolds this necrotic core, separating it from the vascular lumen. The fibrous cap atheroma may produce luminal narrowing and also it may undergone complications, such as rupture, thrombosis, and hemorrhage. Plaque rupture with ulceration is the dominant mechanism that leads to thrombus formation. Atherosclerosis lesions may result in cardiovascular diseases. Thrombotic occlusion of a coronary atherosclerotic plaque commonly causes acute myocardial infarction while embolization from a carotid plaque may result in stroke. The integrity of the fibrous cap is critical to ensure the stability of the plaque. When the fibrous cap is thin, the plaque is prone to erosion and rupture and thin-cap fibroatheromas are vulnerable plaques with high risk of complications. Accordingly, the most common underlying plaque morphology in the coronary arteries of patients dying of acute myocardial infarction or sudden cardiac death is a ruptured thin-cap fibroatheroma with superimposed thrombosis. In early atherosclerosis lesions, calcification may be identified as scattered intimal microcalcifications. This calcification pattern becomes more intense with macrophage infiltration in the fibroatheroma plaque forming the fibrocalcific plaque [9,10].

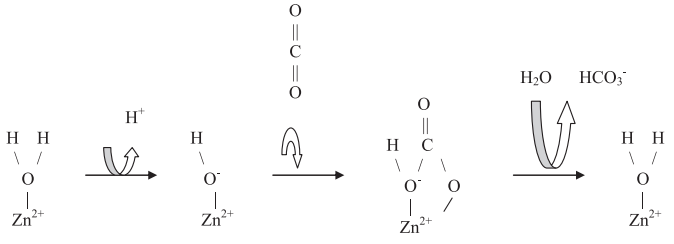

Fig. 1. Carbonic anhydrase reaction: $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$.

Medial calcification is characterized by calcification of the tunica media of the arterial wall with no foam cells associated with the lesion. Medial calcification may occur in large elastic-type arteries (aorta), medium-sized arteries such as kidney arteries, and small arteries (coronary arteries). Medial calcification produces arterial stiffness and reduces arterial compliance, leading to adverse clinical effects including systolic hypertension and left ventricular hypertrophy (Fig. 2) [9,10].

Vascular calcification has been associated with increased risk of cardiovascular mortality due to coronary artery disease in a number of populations [11]. In patients with newly diagnosed type 2 diabetes mellitus, medial arterial calcification is a strong independent predictor of cardiovascular mortality [12]. In a prospective cohort of type 1 diabetes mellitus patients, lower-extremity arterial calcification is an independent correlate of the later presence of coronary artery calcification [13]. In a population-based cohort of individuals who attended health checkups, aortic calcification is independently associated with coronary heart disease risk [14]. In a meta-analysis of prospective studies reporting calcifications and cardiovascular end-points, the presence of calcification in any arterial wall is associated with a 3–4 fold higher risk for mortality and cardiovascular events [15].

Vascular calcification is believed to be an adaptive process initiated by primary damage to the artery wall, either congenital or acquired, although the original causes remain unknown. The molecular mechanisms regulating the development of calcification in human arteries are similar to those that regulate physiological mineralization in bone tissue, being poorly understood. The physiological mineralization process in bones involves hydroxyapatite accretion and begins with the formation of matrix vesicles that sprout from osteoblasts and contain the initiating crystals that are deposited on collagen [16]. In vascular calcification, unidentified stimuli trigger the differentiation of smooth muscle cells into calcifying vascular cells able to generate matrix vesicles and induce calcification with deposit of calcium phosphate complexes similar to bone mineralization [17–19]. A study comparing the transcriptional profile of calcifying vascular cells with that of osteoblasts reveals that smooth muscle cells under an osteogenic stimulus only partially mimic osteoblasts, despite the ability of the transformed muscle cells to induce matrix vesicles-mediated calcification in the arterial wall. Calcifying vascular cells have an overall transcription profile distinct from osteoblasts, although the two cell types regulate identical subsets of extracellular matrix and biomineralization genes. Smooth muscle cells differentiate into calcifying cells and use mechanisms that osteoblasts use to mineralize while preserving their own identity as muscle cells [20].

Similarly to bone tissue, the mineral deposit in human atherosclerotic plaques and medial calcification is partially carbonate-substituted hydroxyapatite (carbonated hydroxyapatite). Chemical analyses have shown that calcium phosphate (hydroxyapatite) amounts to 71% while calcium carbonate is also present in considerable quantity, accounting for almost 9%, being observed particularly in the calcified plaque [21,22]. These data confirm previous findings in which the x-ray diffraction pattern from calcified areas in human blood vessels was similar to that obtained from bone, having been identified as an apatite pattern with crystallites (microcrystalline hydroxyapatite) [23].

Inorganic pyrophosphate (PPi) is a mineralization inhibitor that binds to hydroxyapatite preventing further crystallization. The role of pyrophosphate deficiency in vascular calcification is underlined by heritable disorders caused by mutations in genes that encode enzymes involved in pyrophosphate formation from ATP [24] (Fig. 3).

The transmembrane transporter ABCC6 facilitates ATP egress from cells. Outside the cell, ATP is hydrolyzed to AMP and inorganic