Extracellular Vesicles and Their Relationship with the Uremia

Subjects: Pathology
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Extracellular vesicles (EVs) have been widely investigated for their role in intercellular communication and as potential biomarkers; and could be a promising tool to improve the quality of care in kidney disease patients. Our research group previously demonstrated that the EVs can be related to endothelial dysfunction and are formed when UTs are in contact with the endothelial monolayer. Thus, this review addresses the relationship between these vesicles, cardiorenal syndrome, and uremia.

Keywords: extracellular vesicles ; cardiorenal syndrome ; uremic toxins

1. Introduction

Extracellular vesicles (EVs) have been widely investigated for their role in intercellular communication and as potential biomarkers, particularly in inflammatory pathological conditions. Cardiorenal syndrome results from interrelated heart and kidney injuries, which leads to an accumulation of uremic toxins in the body, especially with the progression of chronic kidney disease (CKD) [1][2][3]. However, the participation of EVs in CRS has not been fully elucidated. Clinical and in vitro studies have shown that uremic toxins induce the formation of EVs [4][5][6][7][8]. In this review, we address the role of EVs in CRS, especially their relationship with uremic toxins and kidney dysfunction. We also discuss the classification of EVs and the main methods for isolating and characterizing EVs, including electron microscopy, proteomics, lipidomics, transcriptome and metabolomics analyses, Fourier transform infrared (FTIR), and Raman spectroscopies, as well as possible use of EVs as biomarkers of cell injury.

2. Cardiorenal Syndrome and Uremic Toxins (UTs)

CRS is a set of diseases with clinical and metabolic consequences triggered by acute and/or chronic heart failure (CRS I and II) or acute and/or chronic kidney disease (CRS III and IV) resulting in injury to the other organs. Failure of both organs can also occur simultaneously as a consequence of a systemic disease (CRS V). Despite the current categorization of the CRS into these five groups, substantial overlap is observed between the different types [9].

Type 2 CRS is characterized by chronic heart failure causing chronic kidney disease (CKD). The underlying mechanisms involve chronic kidney hypoperfusion, increased renal vascular resistance, overactivation of the SNS and RAAS, increased venous pressure, volume overload, endothelial dysfunction and inflammation [10]. This subtype of CRS is very common and has been described in up to 63% of patients with CRS in some reports [11]. The most common mechanisms believed to be involved in the development of type 2 CRS are neurohormonal activation, renal hypoperfusion, venous congestion, inflammation and oxidative stress [10].

UT accumulation can be observed in all types of CRS [3][10][12][13]. Following renal injury, the structural damage in the kidney compromises renal function resulting in a reduction in GFR and/or subsequent increased proteinuria [2], this can cause an increase in UT accumulation in the blood further compounding the functional and structural deterioration of the kidneys and other organs [3]. Although it has been pointed out that this accumulation of toxins causes a primary injury to the kidney, some studies have suggest (i.e., Di Lullo et al.) that it could be considered a type 5 CRS since the uremic compounds can also directly cause damage to the cardiac tissue, featuring a systemic disease [3][10].

The accumulation of medium-sized UT also contributes to renal structural damage, reducing the GFR and cardiac function [14]. Patients with advanced CKD present with low GFR and 1000-fold elevated FGF23 levels [15]. This has adverse effects on the heart via an independent mechanism, promoting cardiac hypertrophy and contractile dysfunction [16][17]. The Pi and FGF23 levels appear to be inter-related by their mechanisms. The Pi levels increase as soon as the renal function

decreases, and plasma FGF23 concentration increases due to significant changes in phosphate or serum PTH concentration. One study considered FGF23 a secondary UT, since it only increases after phosphate accumulation [18]. There are relevant findings regarding Pi and medium-sized UT inducing hypertrophy of the myocardium, hyperplasia of cardiomyocytes, and interstitial fibrosis and vessels [2].

3. The Importance of Extracellular Vesicles in Heart/Kidney Axis and Uremia

The EVs are vesicular nano-sized membrane-enclosed structures composed of a lipid bilayer (such as the cell plasmatic membrane) which transport body cargo such as DNA, RNA, and proteins from their cell of origin and have the ability to physiologically and pathologically influence their cell of origin and other cells [19][20][21]. They can be detected in plasma, urine, and other body fluids of healthy people [22], and their levels are increased in various diseases, mainly reflecting the injury suffered in these tissues [23]. As their composition depends on the pathophysiological and functional state of their cell of origin, they have been studied as potential biomarkers in several diseases, especially cardiovascular [24][25][26], immune [27][28][29][30], cancer [31][32][33][34][35], viral infection, including COVID-19 [36][37], CKD [4][38][39][40][41], and recently in peritoneal dialysis [42][43][44][45][46].

According to the International Society of Extracellular Vesicles (ISEV), EVs are all vesicles released from a cell which can be classified based on their mechanism of formation, mode of release from the cells and size [47][48]. Studies have broadly divided EVs into three main groups: microvesicles (MVs) (also called microparticles), exosomes, and apoptotic bodies.

The role of the UTs in the release of EVs, have mostly been studied in the setting of endothelial dysfunction leading to a release of endothelial-derived microvesicles (EMVs). In vitro and clinical studies observed that PCS was able to induce spreading of EMVs in cell culture as well as increase the levels of EMVs in hemodialysis patients [49]. EMVs also have an important role in patients with ESRD, mainly modulating vasorelaxation, and decreasing endothelial nitric oxide (eNO) release [50]. Favretto et al. observed the formation of different-sized EVs from endothelial cells generated by PCS, IS, and Pi treatments in vitro. In addition, it stimulated cell adhesion markers in PCS and Pi-induced EVs and VCAM-1 expression in PCS and IS-induced EVs [4].

When exposed to an increased hemodynamic load due to physiological stress such as CRS, the heart responds adapting to new operating conditions; however, prior to this adaptation, it responds with a particular communication using EVs to mediate the various cell populations [51][52]. This interaction was previously described by Waldenström et al. when internalization of cardiomyocyte exosomes was observed in fibroblasts and endothelial cells [53]. This study showed the presence of genetic material of cardiomyocytes inside the cytoplasm of other cells types, promoting modification of gene expression. In this context, the EV interaction between cardiomyocytes and fibroblasts was important in the progression of chronic heart failure and is given by the transport of the miR-217 from cardiomyocytes to fibroblasts promoting its proliferation and consequent fibrosis [54]. Cardiomyocytes have also been show to release exosomes internalized by endothelial cells containing miRNAs in order to increase angiogenesis after stress (miR-17,19a,19b,20a,30c,126) [55].

4. Isolation and Characterization of Extracellular Vesicles (EVs)

One of the most used methods for isolating EVs is differential ultracentrifugation (DUC), in which the solution containing the EVs is subjected to several centrifugation steps with increasing speed to pellet the EVs. The isolation of EVs by DUC usually consists of a step with low-speed centrifugation (1000 RCF) to remove cells and larger particles, followed by another intermediate speed spin (20,000 RCF) to collect larger EVs, and finally high-speed ultracentrifugation (100,000 RCF) to isolate smaller EVs [56]. However, it is worth noting that several parameters can affect the type, purity, and yield of EVs, such as rotor type, g-force, centrifugation time, sedimentation rotor angle, and viscosity of the sample [57][58][59][60]. Considering these parameters, possible adaptations to the centrifugation protocol, such as changing the centrifugation time, can improve the separation of the required EVs [58]. For instance, viscous samples need more time and greater ultracentrifugation speed [58].

Mass spectrometry techniques are extensively used in order to determine the proteomic profile of EVs. In summary, EVs are lysed and the proteins are subjected to enzymatic digestion with subsequent separation of the peptides in the mass spectrometer [61][62][63]. The proteomic study of EVs has an important role in the research of biomarkers in EVs that would normally be masked by abundant soluble proteins [43][64].

More recently, the metabolomic study of the content of EVs has gained ground [65]. Some studies have identified significant metabolites in EVs in pathological conditions, such as cancer [66][67]. However, it is necessary to use metabolite extraction protocols and define the analytical platform to study the metabolome of EVs using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and gas chromatography-mass spectrometry (GC-MS) [68][66][69].

Recent studies have also shown that the IR spectroscopy–based protein quantification can be successfully adapted to experimental practice to analyze EVs. In contrast, vibrational spectroscopy presents a reagent-free alternative to traditional colorimetric protein determination assays and demands no special sample preparation to explore EVs [70]. According to results obtained by Paolini et al., FTIR also has the potential to promptly characterize EV subpopulations [71], suggesting it as an attractive complement or alternative method for understanding EVs in healthy and pathological situations.

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