Vascular dysfunction in preeclampsia
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Chapter 3

Microparticles in cardiovascular diseases


Submitted.
Abstract

Microparticles are membrane vesicles released from many different cell types. There are two mechanisms that can result in their formation, cell activation and apoptosis. In both these mechanisms different pathways are involved in microparticle generation. Microparticle generation seems to be a highly regulated process that results in formation of microparticles that vary in size, phospholipid and protein composition. Microparticles have a potent pro-inflammatory effect, they promote coagulation and they affect vascular function. Since all these processes are involved in the pathogenesis of cardiovascular disease and microparticle numbers in venous blood are altered in many cardiovascular diseases, a role for microparticles in the pathogenesis of cardiovascular diseases is likely. Although the so far gathered information points towards a causal role of microparticles in these diseases, additional evidence is required to confirm this. Clarification of the microparticle composition and the mechanisms involved in exertion of their effects will hopefully supply us with this evidence and enable us to develop additional intervention strategies for prevention and treatment of cardiovascular diseases.
1. Introduction

Microparticles were first described in 1967 when Wolf reported shedding of membrane fragments after activation of platelets in vitro [152]. He called these fragments “platelet dust”. This “dust” contained vesicles, smaller than 0.1 μm in diameter, which promoted coagulation and were also present in plasma. In the past decades it has become apparent that many cell types can release microparticles and that these microparticles may not just be side effects of cellular processes, but may be involved in physiology and pathophysiology. In vitro, the release of microparticles from endothelial cells, vascular smooth muscle cells, platelets, leukocytes, lymphocytes and erythrocytes has been shown and the occurrence of these microparticle populations has been demonstrated in the venous blood of both healthy volunteers and various patient groups. The numbers and composition of the various microparticle populations are disease dependent, but the impact of these changes on their in vivo function is insufficiently known. Microparticles have been implicated to play a role in inflammation, coagulation, and vascular function. In this review, we will summarize recent information on microparticle formation, composition, and – most important - their putative physiological and pathological functions in cardiovascular diseases. Furthermore, we will discuss the evidence that some currently used therapies may in fact partially exert their effects via the blockade of microparticle formation.

2. Microparticle formation

There are two well-known cellular processes that can lead to the formation of microparticles, cell activation and apoptosis. At present we do not know whether cell activation and apoptosis lead to the formation of similar microparticles, in terms of their size, lipid and protein composition and their (patho-)physiological effects. There are, however, differences in the mechanisms resulting in their formation. The processes thought to be involved in microparticle formation during cell activation and apoptosis are presented schematically in figure 1.

2.1. Cell activation

The cellular activation-associated formation of microparticles can be induced by many agonists, dependent on the cell under study (figure 1, left panel). Platelets, for instance, can be activated by thrombin, calcium ionophore A23187, adenosine diphosphate (ADP) plus collagen, the terminal complement complex C5b-9 or shear stress [153-165]. Other cells can also be activated by e.g. bacterial lipopolysaccharides, cytokines, such as TNFα or IL-1, C5b-9 complex or by hydroperoxide [166-171]. In general, the release of cell activation-associated microparticles is time- and calcium-dependent. The shedding starts within minutes after stimulation of cells with agonists [158,160,172]. As one of the first signs of cell activation, cytosolic calcium concentrations rise as part of the overall activation process [161,163,169], but also and especially at the site of vesiculation [173]. Preventing this calcium elevation by chelating extracellular...
calcium with EGTA prevents vesiculation [169]. The cytosolic calcium increase leads to several downstream processes in the cellular activation, including (in)activation of several kinases, phosphatases and the protease calpain [161,163,169,174-177].

Microparticle formation requires the breakdown of the membrane skeleton, the subcellular system that provides the cell membrane with structural stability [178]. This membrane skeleton mainly consists of actin, and also of vinculin, talin and other muscle-like proteins. The exact interaction between the cell membrane and the membrane skeleton, which prohibits microparticle formation, is currently unknown. Part of the breakdown of the membrane skeleton is effected by the calcium-dependent protease calpain, which degrades talin [169]. This is one of the direct pathways through which the increased cytosolic calcium is involved in facilitation of microparticle formation.

Microparticle formation is also in some way linked to the glycoprotein (GP) IIb-IIIa complex. This complex, in its active conformation, functions as the main fibrinogen receptor on the platelet membrane. RGD, the peptide sequence within the fibrinogen molecule that binds to the GP IIb-IIIa complex, inhibits microparticle formation induced by several agonists [160]. This implies that the GP IIb-IIIa complex is involved in the cell activation-associated microparticle formation.

**Figure 1. Schematic representation of mechanisms involved in microparticle formation during cell activation and apoptosis.**
Microparticles in cardiovascular diseases

In platelets (figure 1, left panel). The role of GP IIb-IIIa in platelet microparticle formation is supported by studies with platelets from patients with Glanzmann's thrombasthenia. Their platelets have reduced amounts or complete absence of functional GP IIb-IIIa and an impaired ability to vesiculate [160].

2.2. Cell apoptosis

Blebbing of membranes is a well-known phenomenon of cells undergoing apoptosis [174,179]. Such blebs may differ from microparticles formed by cell activation in size, lipid and protein composition and (patho-)physiological effects. Apoptosis is characterized by cell contraction, dynamic membrane blebbing and DNA fragmentation. The contractile force, generated by actin-myosin cytoskeletal structures, is thought to drive the formation of membrane blebs [180] (figure 1, right panel) [181]. Occurrence of apoptotic membrane blebbing is dependent on the function of the rho-associated kinase, ROCK I [174]. ROCK I promotes generation of contractile force by contributing to increased actin-myosin force generation and coupling of actin-myosin filaments to the plasma membrane [182]. During apoptosis ROCK I is cleaved by activated caspases, which results in the generation of an active truncated kinase. This active kinase drives cell contraction and membrane blebbing [174]. ROCK I activity and, as a consequence, membrane blebbing are required for redistribution of fragmented DNA from the nuclear region into the membrane blebs and apoptotic bodies [174]. Thus, microparticle formation during apoptosis results from ROCK I activity and the resulting disruption of the membrane skeleton structure, and results in formation of microparticles that can contain fragmented DNA (figure 1, right panel).

3. Microparticle composition

Microparticles consist mainly of lipids and proteins. Each of these constituents may differ between microparticles, depending on their cellular origin, the cellular process triggering their formation and on other factors.

3.1. Lipids

Microparticles are surrounded by a phospholipid bilayer. In resting cells the various phospholipid species are distributed asymmetrically in the phospholipid bilayer. This asymmetrical phospholipid distribution is usually disturbed during microparticle formation [183]. One of these phospholipids, phosphatidylserine (PS), is located almost exclusively in the cytosolic leaflet of the cell membrane of resting cells. Upon activation or during apoptosis PS redistributes and becomes present also in the outer leaflet, which contacts the milieu extérieur, thereby providing the microparticle with a negatively charged - and thus procoagulant - surface [184].

Only limited information is available on the phospholipid composition of microparticles in health and disease. Weerheim and coworkers reported the phospholipid composition of microparticles in the venous circulation of healthy humans [185]. They consisted mainly of phosphatidylcholine (PC, approximately 60%) and smaller amounts of e.g. sphingomyelin (SM),...
phosphatidylethanolamine (PEa) and PS were present [185]. Although microparticles in the venous circulation of healthy volunteers are mainly derived from platelets [186], the phospholipid composition of these microparticles and composition of the platelet membrane clearly differs. This is probably due to the presence of microparticles from other cells and/or from selective shedding of phospholipids into microparticles. Fourcade and coworkers reported that microparticles from synovial fluids of inflamed joints of patients with arthritis contain PC, PEA, SM, and lysophospholipids (all 20-25%) and small amounts of PS [165]. This composition clearly differs from that of the microparticle population in venous blood from healthy humans [185]. Microparticles in the synovial fluids are mainly derived from leukocytes [187] and circulating microparticles in healthy humans mainly from platelets. This may indicate that the phospholipid composition of microparticles from different cell types differs, or alternatively, that inflammatory stimuli can produce microparticles with varying phospholipid composition. Huber and coworkers recently reported the presence of oxidized phospholipids in microparticles released from endothelial cells exposed to an oxidative stress stimulus, and in microparticles exposed to oxidative stress after their formation [172]. Oxidized phospholipids were not present in microparticles released from cells stimulated with a non-oxidative stimulus [172].

Thus, the phospholipid composition and the phospholipid oxidation status may differ between microparticles from different parent cells or subjected to different conditions or stimuli during their formation.

3.2. Proteins

Microparticles expose membrane antigens that are specific for the “parent cell” they originate from, which enables the determination of their cellular source [188]. However, microparticles may also expose molecules that have been upregulated by cell activation or apoptosis [167]. For instance, several antigenic determinants of microparticles from TNF-stimulated cultured endothelial cells, e.g. adhesion molecules, were also present on resting cells, but these microparticles also exposed antigenic determinants that were upregulated by activation [167]. Furthermore, microparticles can expose components that are derived from granules within the cell, which are not necessarily exposed on the surface of the non-activated “parent cell”. For example, microparticles from platelets that secreted their granules upon activation, expose molecules derived from these intracellular granules such as P-selectin and GP 53 [154,162]. These microparticles are also highly enriched in α-granule-derived factor Va [162]. Similar observations were done in T cell-derived microparticles, which exposed GP 53 from endocytic origin [189].

Microparticle shedding must be a well-regulated process, since major differences can occur in antigen exposure and densities between “parent cells” and microparticles derived there from [189,190]. For example, T cell-derived microparticles lack the proteins CD28 and leukocyte common antigen (CD45), which are among the most abundantly present proteins of the parent cell membrane [189]. Stimulation of platelets with complement complex C5b-9 produces microparticles that, compared to the platelets, are highly enriched in the C9-neoantigen [162] and have a 1000-fold higher surface density of C5b-9, suggesting that these microparticles are shed
from the site of insertion of the C5b-9 complex [162]. Also, erythrocyte-derived microparticles are specifically enriched in various antigens and receptors [191,192]. The fact that the sorting process of proteins into the microparticles is a complex phenomenon is further illustrated by the fact that some of the proteins present in lipid rafts-specific subdomains of the membrane that are enriched in cholesterol- and sphingomyelin as well as several proteins - of the erythrocyte membrane are transferred into microparticles, while others are not [190].

The sorting process of proteins into microparticles is actually even more complex, because their composition also depends on the agonist that triggers microparticle generation. T cells produce microparticles that are enriched in CD3e- and ζ-chains only upon activation of the T cell antigen receptor and not upon activation by ionomycin plus p-methoxyamphetamine hydrochloride [189]. Microparticles from thrombin- or collagen-activated platelets expose GP IIb-IIIa complexes that bind fibrinogen, in contrast to microparticles produced by platelets incubated with C5b-9, which do not bind fibrinogen [154]. However, it should be kept in mind that even microparticles formed by the same cell population in response to a single agonist can still form a heterogeneous population. For instance, microparticles released from platelets after stimulation with serum from patients with heparin-induced thrombocytopenia are heterogeneous in size and in GP IIb-IIIa exposure [193].

Thus, microparticles vary in size, phospholipid and protein composition and therefore probably in their functional capacity and activity. The shedding seems to be a well-regulated and selective process, thereby creating differences in microparticle characteristics.

4. Microparticle function

Microparticles are present in the circulation of healthy humans. We described the numbers and cellular origin of microparticles in the blood of healthy men [186] and women (see chapter 5). Circulating microparticles were mainly derived from platelets, but also from erythrocytes, leukocytes and endothelial cells [186,194]. These microparticles initiated thrombin generation \textit{in vitro} [186] (see chapter 8). Microparticles have also been studied in various disease states, in which their numbers, cellular source and composition proved altered. These observed differences are likely to affect their putative functions. Although many aspects of microparticle function are still unclear, a picture develops in which microparticles play an important role in inflammation, coagulation, and vascular (dys-)function. Theoretically, microparticles may have different (patho-)physiological functions, namely 1) enriched shedding of membrane components from the parent cell which can then be transported to other cells, 2) direct activation of inflammation, coagulation or vascular function by components on the microparticle surface and 3) indirect activation of these processes via their activating effects on various cells. Since inflammation, coagulation and vascular function are all involved in the pathogenesis of cardiovascular diseases, we will discuss how microparticles can be involved in these disease processes (see figure 2) and illustrate their putative roles.
4.1. Inflammation

4.1.1. In vitro evidence

An increased inflammatory response is involved in the pathogenesis of cardiovascular diseases. Adhesion of monocytes and neutrophils to the endothelium is an early event in vascular inflammatory syndromes and, together with the subsequent transendothelial migration of the leukocytes, a crucial step in the development of atherosclerosis [195]. Adhesion molecules exposed on endothelial cells attract various classes of leukocytes to the vascular wall. Microparticles expose cell-specific adhesion molecules on their surface. For instance, endothelial cell microparticles, derived from resting or TNF-α-stimulated cells, expose the adhesion molecules intercellular adhesion molecule-1 (ICAM-1), E-selectin, vitronectin-3 and platelet endothelial cell adhesion molecule-1 (PECAM-1) [167]. Furthermore, microparticles trigger exposure of adhesion...
molecules or their ligands on endothelial cells and leukocytes, thereby stimulating interactions between these cells, as summarized in table I. Such interactions promote the inflammatory response (see figure 2).

4.1.2. In vivo evidence

There is some evidence for a role of microparticles in inflammatory processes available from in vivo studies. Mesri and coworkers described a heterogeneous microparticle population in healthy humans, of which the numbers could be doubled by administration of N-formyl-Met-Leu-Phe, an inflammatory stimulus. The resulting microparticle population contained both leukocyte- and platelet-derived microparticles [201]. The leukocyte microparticles in this population induced cultured endothelial cells to produce IL-6 and monocyte chemoattractant protein-1 and to expose tissue factor (TF) [201]. During various systemic inflammatory conditions, microparticle numbers are increased in the systemic circulation, as summarized in table II.
Table II. Diseases in which total microparticle numbers or numbers of subgroups of microparticles in the venous circulation are increased.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Increased microparticles</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute coronary syndromes</td>
<td>Total, endothelium, platelet</td>
<td>[202,203]</td>
</tr>
<tr>
<td>Arteriosclerosis obliterans</td>
<td>Platelet</td>
<td>[204]</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Total and platelet</td>
<td>[205,206]</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Platelet</td>
<td>[205]</td>
</tr>
<tr>
<td>Idiopathic thrombocytopenia</td>
<td>Platelet</td>
<td>[207,208]</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>Endothelium</td>
<td>[167]</td>
</tr>
<tr>
<td>Paroxysmal nocturnal hemoglobinuria</td>
<td>Total and platelet</td>
<td>[209]</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>Leukocyte</td>
<td>[194]</td>
</tr>
<tr>
<td>Sepsis</td>
<td>Leukocyte</td>
<td>[210,211]</td>
</tr>
<tr>
<td>Systemic inflammation after trauma or sepsis</td>
<td>Platelet</td>
<td>[212]</td>
</tr>
<tr>
<td>Uremia</td>
<td>Platelet</td>
<td>[205]</td>
</tr>
</tbody>
</table>

4.1.3. Microparticle components involved in inflammation

Oxidized phospholipids may be the biologically active components of microparticles that cause monocyte adherence to endothelial cells and neutrophil activation [172]. Patel and coworkers identified these oxidized phospholipids in microparticles from endothelial cells, activated with peroxide, which facilitated intercellular adhesion [168]. As previously mentioned, these oxidized phospholipids are present in endothelial microparticles subjected to oxidative stress during or after formation, but not in microparticles released after a non-oxidative stimulus [172]. Furthermore, apoptosis is accompanied by oxidative stress [213] and microparticles from endothelial cells undergoing apoptosis contain oxidized phospholipids [172]. Oxidative stress and apoptosis are well-recognized phenomena in many cardiovascular diseases, such as cardiomyopathy, myocarditis, acute myocardial infarction, atherosclerosis and preeclampsia (reviews see [179,214,215 and 216]).

Furthermore, oxidized phospholipids in low-density lipoproteins are implicated in the pathogenesis of atherosclerosis (for reviews see [214 and 217]). Therefore, the occurrence of oxidized phospholipids in apoptotic microparticles and in microparticles formed in the presence of oxidative stress may be an important mechanism in the pathogenesis of these diseases. Oxidized phospholipids exert their actions through platelet activating factor (PAF) receptors [168,218], which are exposed on both endothelial cells and leukocytes [219]. The exact pathways through which the effects of oxidized phospholipids are accomplished after their interaction with the PAF receptor are not yet clarified, but may be similar to the actions of PAF when it binds to the PAF receptor, i.e. internalisation of the complex and increase of intracellular calcium
concentrations [220,221]. However, another possible mechanism is the delivery of arachidonic acid by microparticles to endothelial cells, monocytes and platelets [157,196]. Since arachidonic acid is a component of oxidized phospholipids the two above proposed mechanisms could in fact be part of the same mechanism.

Thus, considering the above described evidence for microparticle-leukocyte-endothelial cell interactions, the altered numbers of circulating microparticles in patients with inflammatory and cardiovascular diseases or with cardiovascular risk factors and the recognition of a possible role for microparticle phospholipids and their oxidation status in these processes, provide evidence that microparticles are actively involved in inflammatory processes and thus in cardiovascular diseases.

4.2. Coagulation

4.2.1. In vitro evidence

Patients with cardiovascular diseases have an increased risk of thrombosis, which can manifest itself as acute myocardial infarction or stroke [222]. There is substantial in vitro evidence for microparticle involvement in activation of the coagulation system. Coagulation requires not only (activated) coagulation factors and calcium ions, but also membranes exposing negatively charged phospholipids, such as PS. Exposure of PS facilitates binding of (activated) coagulation factors to the membrane, thereby enabling the formation of tenase- and prothrombinase-complexes. Subsequently, blood coagulation can start, especially when TF - a transmembrane protein that initiates coagulation through the extrinsic coagulation pathway - is exposed.

Microparticles have a negatively charged phospholipid surface [183], readily bind activated coagulation factors [154,158,162,223] and expose TF in various conditions [166,171,210,224-226]. Both in vitro prepared and in vivo generated microparticles initiate and support thrombin generation in vitro [167,184,186,187,210,211,224,226]. Furthermore, infusion of artificially prepared phospholipid vesicles supports the development of severe disseminated intravascular coagulation in primates [227], and infusion of these vesicles in pregnant rats induces placental congestion and growth restriction in the offspring [228].

Besides the thus far described direct effects of microparticles on the coagulation system, microparticles may also indirectly promote coagulation. For instance, purified P-selectin or P-selectin-exposing platelets trigger the expression of TF on monocytes [229]. Since P-selectin is often present on platelet-derived microparticles, also these microparticles are likely to be involved in induction of TF expression on monocytes (see figure 2).

4.2.2. In vivo evidence

The evidence that microparticles actually contribute to coagulation in vivo is mainly circumstantial. First, microparticle numbers are elevated in different types of disease involving hypercoagulation, such as idiopathic thrombocytopenia, paroxysmal nocturnal hemoglobinuria, lupus anticoagulant and acute coronary syndromes (see table II). Second, microparticle generation is reduced in several bleeding disorders, such as Scott Syndrome [154], Castaman's
defect [230] and Glanzmann’s disease [160]. Platelets from patients with the Scott syndrome and Glanzmann’s disease have a decreased microparticle generating capacity in response to agonists [154,160] and have a reduced numbers and function of inducible factor Va receptors [154]. Third, microparticles expose TF in several clinical conditions that are strongly associated with hypercoagulation, such as pericardial wound blood [226], the blood of patients with disseminated intravascular coagulation [211], and synovial fluid from inflamed arthritic joints [187].

Since the occurrence of hypercoagulation is one of the characteristics of cardiovascular diseases, and altered numbers and procoagulant behaviour of microparticles were reported in several cardiovascular diseases, microparticles may play a causal role in the development of hypercoagulation in cardiovascular disease.

4.3. Vascular function

4.3.1. In vitro evidence

Diminished vascular function, especially endothelial dysfunction, has been reported in many cardiovascular diseases. Well known examples are the pathogenesis of cardiac failure [231], atherosclerosis [232], acute coronary syndromes [233], hypertension [234], and preeclampsia [4]. Several studies reported the effects of microparticles on endothelial cell activation and function in vitro. Platelet microparticles, isolated from human platelets stimulated with various agonists, induced cyclooxygenase (COX)-2 expression and PGI₂ production in cultured endothelial cells [157]. The COX-2 expression was initiated by the arachidonic acid fraction of microparticles [157]. Recently, Boulanger et al. described that microparticles from patients with acute myocardial infarction diminished endothelium-dependent relaxation in isolated bioassay arteries [235]. In contrast, microparticles isolated from the venous blood of patients with non-ischemic chest pain had no such effect [235]. This is the first demonstration of a direct effect of microparticles on vascular function. Recently, we demonstrated that also microparticles from venous blood of women with preeclampsia diminished endothelium-dependent relaxation in isolated resistance arteries [236] (see chapter 6 and figure 2).

4.3.2. In vivo evidence

Only circumstantial evidence is presently available for a role of microparticles in vascular dysfunction in vivo. Microparticle numbers are elevated or the composition of the microparticle population is altered in cardiovascular diseases that are characterized by endothelial dysfunction, such as acute coronary syndromes, hypertension, atherosclerosis and preeclampsia (see table II). In atherosclerosis high levels of presumably apoptotic microparticles, mainly derived from monocytes and lymphocytes, are also present in the atherosclerotic plaques [237].

4.3.3. Microparticle components

One of the mechanisms that could mediate the above-described effects on the vascular wall could again be oxidized phospholipids, especially since oxidized PC induces endothelial dysfunction in isolated bioassay arteries [238].
5. Are microparticles cause or consequence of cardiovascular diseases?

In the previous paragraphs we summarized the evidence that microparticles are likely to contribute to the pathogenesis of cardiovascular disease; their potent pro-inflammatory effect, their promotion of coagulation, and their effect on vascular function. However, it remains to be established whether microparticles play a causal role in the pathogenesis of these diseases or whether they are a consequence of the disease. In the previous paragraphs several facts were discussed that indicate an active, causal role of microparticles in the pathogenesis of cardiovascular diseases. The strongest evidence for this hypothesis is provided by the studies that showed a direct effect of microparticles on endothelial function. Furthermore, the fact that \textit{in vivo} and \textit{in vitro} prepared microparticles induce increased adhesion of leukocytes to endothelial cells, trigger cytokine production and expose TF or P-selectin, strongly suggests an active role of microparticles in inflammation and coagulation. However, increased microparticle numbers can also be a result of inflammation, hypercoagulation or vascular dysfunction. For instance, cytokines as well as thrombin can trigger microparticle generation [167,171] or enhance the already existing microparticle generation [154,155,159,160,198,204,223,239].

6. Effects of currently used therapies on microparticles

The recognition of a role of microparticles is not only important for our understanding of the pathogenesis of cardiovascular disease, but also has important implications for prevention and treatment of these diseases. Some currently used therapies are known to affect microparticle generation. For instance, abciximab (ReoPro®), a GP IIb-IIIa receptor antagonist that is currently used as an antiplatelet drug in prevention of ischemic complications after percutaneous coronary intervention, also blocks platelet vesiculation [240]. Treatment of patients suffering from a transient ischemic attack with calcium channel blockers also decreased microparticle generation [241]. Furthermore, treatment of patients with congestive heart failure with vitamin C decreased the number of circulating microparticles [242]. In this respect, prevention of generation of oxidized phospholipids in microparticles may also be important.

Thus, there are some therapies that are currently used for treatment of diseases that also affect microparticle numbers. Whether these effects on microparticles contribute to the exertion of the effect of the therapies or are only side effects remains to be established. While research into the role of microparticles in cardiovascular disease progresses, more of these effects on microparticles will probably be discovered and may prove useful in prevention or treatment of these diseases.
7. Concluding remarks

In this review we have discussed the current knowledge available on microparticle formation, composition and function. There is considerable evidence that microparticles play a role in the processes of inflammation, coagulation and vascular function, processes that are all involved in the pathogenesis of cardiovascular diseases. Future studies are needed to provide additional evidence whether the role of microparticles in these disease processes is indeed a causal one, as is suggested by the so far gathered evidence. Clarification of the microparticle composition and the underlying mechanisms involved in exertion of the effects of microparticles will hopefully supply us with this evidence and enable us to develop additional intervention strategies for prevention and treatment of cardiovascular diseases.