Microparticle Function

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Abstract

Microparticles are emerging as an integral part of the vascular system. The microparticles are derived from stimulated or apoptotic endothelial cells, platelets, and leukocytes. They are involved in coagulation and regular cell function. Excessive numbers of microparticles contribute to artherosclerosis, chronic renal failure, and metabolic syndrome. Microparticle levels may be reduced by medications, relieving symptoms for awhile. Studies are being done to predict disease by counting microparticles. Further research must be done to understand and make use of microparticles.

Introduction

Endothelial microparticles (EMPs) are small (> 1 μm in diameter), "non-nucleated phospholipid vesicles shed from the endothelial cell membrane in states of endothelial activation or apoptosis and express on their cell surface various antigens specific to the state of their parental endothelial cell" (Weber et al 2011). They were thought to have been markers for endothelial dysfunction. Recently, studies have shown that increased circulating microparticles (MP), which now include platelet microparticles (PMP) and leukocyte microparticles (LMP), also have a significant effect in contributing to endothelial dysfunction in the human body. They both mark and augment pre-existing conditions. MP's contribute to: apoptosis, thrombosis and arterial stiffness. Patients with kidney disease, obesity, and pulmonary hypertension are more susceptible to higher levels of microparticles than their healthy counterparts. The increased numbers may be caused by triggers, released as a byproduct of the diseases. High MP levels may lead to cardiovascular disease, one of the leading causes of death in the United States. This article will explore what affects and reactions microparticles may cause (Morel et al 2006). In addition, it has been found that MP's can be used as a biological tool. While microparticles have many functions based on origin, this paper will be focusing on the MP's that affect endothelial dysfunction, mainly: EMP, PMP, and LMP.

Methods

Searches were done primarily from PubMed. Once an article was deemed helpful, the PubMed ID was put into Touro's library ID search which gave access to the article through EBSCO, Proquest Medical Library, and Medline. Articles were also taken from Google Scholar. The first articles were found using the following methods. Keywords: microparticles, endothelial microparticles, platelet microparticles, microparticles and coagulation, microparticles and pulmonary disease, microparticles and atherosclerosis, microparticles and renal failure, mircroparticles and obesity, . Articles were filtered within the past 10 years, peer-reveiwed, clinical trial, and meta-analysis. Sources found in these articles were also used as references when further clarification was needed.

Definition

Microparticles are small membrane-bound vesicles that are released from the cell during cell stimulation or cell death. Electron microscopy techniques allowed Wolf to identify microparticles released from platelets, a particle that had been speculated upon but had never been seen. He further discovered that they were released from the cell after being triggered by chemicals such as cytokines, thrombin, and endotoxins (Budaj et al 2012). Chrinios et al (2005) believe that MP's are triggered by cell activation due to the expression of tissue factor, the main trigger for thrombin generation.

MP's can be found in multiple places, including blood, urine, ascetic fluids, and synovial fluids (Bundaj et al 2012).Contained on the EMP cell surface are many proteins including vascular endothelial cadherin, platelet endothelial cell adhesion molecule-1, endothelial NO synthase (also found on PMP), and vascular endothelial growth factor receptor. The last protein is particularly important in tagging EMP's in the blood (Dignat-George and Boulangar 2011).

Different types of Microparticles

The three groups of MP's are classified by their origins. EMP's are derived from the membrane of the vascular endothelial cell. For this reason, microparticles are mostly studied along with their effect on vascular and cardiological diseases. EMP's are involved in endothelial cell function. Chrinios et al (2005) have found that EMP's "express many receptors of the parent endothelial cell" and can help thrombin generation through the tissue factor pathway. Tissue factor is the main cellular initiator of blood coagulation. During EMP production, phosphatidylserine is released, an important molecule that enhances the procoagulant activities of tissue factor. In addition to coagulation and thrombosis, EMP's contribute to other factors of endothelial dysfunction as well. This includes changes in the nitric oxide (NO) synthase expression and local prostacyclin synthesis (Amabile et al 2008). These two molecules will be discussed in further detail below.

PMP's and LMP's are now emerging in new studies of MP's. The studies have shown that they are involved in coagulation, but they are still being researched. PMP's are frequently counted by the P-selectin count, a recognized marker of platelet activation in aggregation between platelets, monocytes, and neutrophils.The coagulation process will generally favor the seeding of LMP followed by PMP and EMP aggregation (Chirinos et al 2005).

Release and Inhibition

Under normal conditions, vessel homeostasis is controlled by

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Under normal conditions, vessel homeostasis is controlled by the intact endothelial cell monolayer which has anti-inflammatory, anti-atherogenic, and anti-thrombotic properties. It is maintained by low continuous cell regeneration. The endothelial activation remains local, low-grade, and reversible. Circulating MP's are barely detectable due to their low count. However, in case of injury cells may respond with apoptosis and MP generation along with other factors leading to a pro-coagulant state (Chironi et al 2009).

One hypothesis for the release of MP's may be that they are released as a way of preservation. The cell will attempt to evade phagocytosis by releasing the phosphatidylserine and caspase 3, both signals that alert the phagocyte. By shedding these factors, phagocytes will not take notice of the cell (Boulanger et al 2006). Another theory suggests that MP's are released in response to certain signals, such as tumor necrosis factor-α; a cytokine. A study has shown that EMP release istriggered by tumor necrosis factor-α and subsequently increased the release of ICAM-1, a T-cell. This allowed a paracrine loop, boosting the endothelial response to inflammation (Dignat-George and Boulanger 2011).

Microparticles are produced in response to changes within the body as well. MPs derived from platelets, endothelial cells, and erythrocytes are produced in response to metabolic perturbation. The monocyte derived MP is produced as a subsequent step in response to more severe conditions, such as diabetes and cardiovascular disease (Helal et al (2012). Oxidative stress has been shown to be a significant factor in MP release. Reactive oxygen species have been shown to reduce the availability of NO, which is a mitochondrial stabilizer, the release of cytochrome c, and caspase activation. These contribute to endothelial dysfunction, leading to the release of EMP's (Morel et al 2003).

Studies have shown that vascular endothelial-cadherin, an adhesion molecule found at junctions between endothelial cells, regulates many cell processes including cell proliferation, apoptosis, and modulation of vascular endothelial growth factor receptor functions (Petzelbauer et al 2000). A study attempted to stimulate in vitro MP release. It was reported that apoptotic and activated cells respond differently depending on the stimulus. Cells were stimulated while deprived of endothelial growth factor. Findings indicate that renal endothelial cells release certain EMP's in larger amounts from apoptotic cells than cells activated by tumor necrosis factor. In contrast, other strains of EMP are significantly higher when activated by tumor necrosis factor than apoptosis. This study also shows that MP's are phenotypically different and may lead to further studies of different types of EMP's in response to different injuries (Jimenezet al 2003).

Conversely, the MP's also have an inhibition cycle in place. Firstly, endogenous NO dampens the release of EMP on stimulation with C-reactive protein by a mechanism involving tetrahydrobiopterin. Studies have also shown the importance of the Rho kinase pathway (figure 1). While its importance is understood in EMP release, the exact mechanism has not yet been reported.

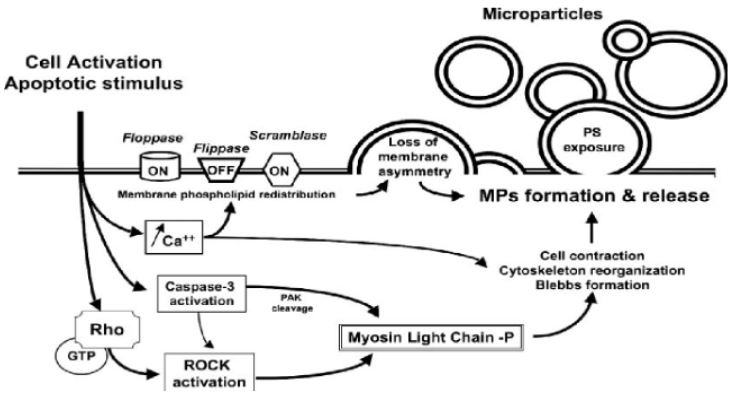

Figure 1: EMP release pathway. Release is stimulated by an increase in Calcium or the Rho kinase pathway (still under study). This causes an imbalance in the membrane or cytoskeleton restructure leading to MP release. Source: Boulangar et al., 2006

The Rho family is an important regulator in cell adhesion and cytoskeleton. The Rho GTPases attach themselves onto the cell membrane and cause subsequent activation of the Rho-kinase (Tramontano et al 2004). This activation reduces the stability of the endothelial NO synthase pathway (Davignon and Ganz 2004). An in vitro study showed that statins anti-inflammatory property is a result of Rho-kinase pathway inhibition. It can be inferred from here that MP's are released in response to inflammation and may be suppressed by the statins (Tramontano et al 2004).

Formation

A cell membrane at rest will prominently display inward translocation (flip). During this time, phosphatidylserine and phosphatidylethanolamine are stored in the inner leaflet of the cell membrane. At stimulation, the molecules will reverse to outward translocation (flop). At hemostasis, aminophspholipids are stationed on the inner leaflet and neutral phospholipids stationed on the outer leaflet. To control the asymmetric distribution, the flop enzyme is only activated by high levels of calcium. During flop, there is a swift movement of the aminophospholippase to the outer leaflet. This causes an extreme imbalance between the inner and outer leaflets (Baron et al 2012). To resolve the imbalance, the cell will begin membrane budding. Membrane budding causes the formation of membrane blebs (figure 1). The cell membrane must undergo changes in membrane lipids, cytoskeleton reorganization, and increase in intracellular calcium (Diehl et al 2011)

The buds cannot retain the symmetric membrane found on the parent cell. They are asymmetric and carry their annexin v (an anticoagulant protein) on the outward surface of the membrane (Kornak and Schuppan 2012). The buds are microparticles containing phosphatidylserine , a molecule that causes coagulation. This feature exposes more procoagulant molecules to the bloodstream proving that MP's are necessary

to achieve the required optimal environment for thrombin generation (Morel et al 2006). However, the presence of phosphatidylserine does not always mean they were released by MP's. MP release may be regulated by the level of intracellular calcium, significantly affecting other studies that used phosphatidylserine as a measurement (Boulangar et al 2006). Phosphatidylserine binds to the annexin-V. It can be inferred from here that this action causes less annexin V to circulate, allowing the thrombus to form (Dignat-George and Boulangar 2011).

Apoptotic cells differ from activated cells in protein and lipid composition. As opposed to cell stimulation, apoptotic cells release DNA fragments to microparticles with the help of an ezyme. The function of the genetic material has not yet been determined. However, certain studies have shown that mRNA transfer from the EMP to other endothelial cells promotes angiogenesis after protein kinase B and NO synthase expression. Blebbing occurs rapidly after the cell enters the apoptotic process. The vesicle formation depends on actin molecules within the cell, regulated by Rho kinase I activation (figure 1). The Rho kinase is needed to relocate the DNA fragments to the MP. Cell activated MP's can be identified by the proteins they contain. The MP's protein will retain traces of the original cell and the stimulus that triggered the release (Boulangar et al 2006). MP's were found to communicate through the use of antigens on the membrane surface. They are able to transfer the antigen to the other cells, thus able to transform the biological function of the cell. (Budaj et al 2012).

Coagulation Pathway

Thrombus Growth

According to a study done by Muller, PMP's are the main source of coagulation, with EMP's and LMP's supplementing. MP's are also known to have cytoplasmic effectors such as selectins, arachidonic acid, and thromboxine which are able to promote prothrombotic responses (Muller et al 2003). It has been shown that PMP's are 100 times more procoagulant than activated platelets, showing the importance of the PMP in a blood clot. PMP's contain arachidonic acid, a vasodilator, increasing aortic tone (Pfister 2004). MP's are then able to transfer their procoagulant potential to the target cell. For example, studies have shown PMP's can bind to and deliver the fibrinogen to the thrombus, promoting coagulation. Interactions between the MP's and monocytes promote mRNA expression of tissue factor, which is a major coagulation effector. EMP's and LMP's are released after activation by bacterial lipopolysaccharides, aggregated low-density lipoproteins, and reactive-oxygen species. Studies have shown that intracellular calcium at the site of vesicle formation is a critical step for MP release (Boulanger et al 2006).

P-selectin is an integral aspect of a thrombus. The P-selectin molecule is an adhesion molecule that is necessary for tissue factor accumulation and incorporation of the leukocytes into the thrombus. The molecule is generally expressed at platelet and endothelial cell surfaces. The buildup of cell-derived tissue factor correlates to the large number of MP's present before the leukocyte-thrombus interaction. In a study involving lab rats with hemophilia A, it was found that soluble P-selectin promoted the shedding of leukocyte-derived MP's carrying tissue factor. This product helps with hemostasis. Studies have shown that MP levels increase as people age. A study done with P-selectin glycoprotein ligand-1in mice has indicated that the MP level is dependent on the contribution of P-selectin, showing the importance of the P-selectin glycoprotein ligand pathway. If the P-selectin was decreased, as in this case, the MP's were decreased as well (Hrachovinova et al 2003).

Stability of the Thrombus

The thrombus may be stabilized by fibrin formation, induced by P-selectin. However, P-selectin glycoprotein ligand interactions are generally noted by their unstable rolling and other stabilizations are needed. Cytoadhesions, a β integrin on the LMP, contribute to stabilization of the thrombus. EMP's exhibit unusually large von Willebrand glycoproteins that promote platelet aggregation. The thrombus is regulated by phagocytes in order to prevent inflammatory responses. Little is known about MP clearance within the thrombus. However, studies on mice have shown that oxidized LDL can either blunt or saturate phagocytosis of apoptotic cells within the thrombus. The oxidized LDL interferes with recognition of MP phosphatidylcholine moieties by macrophage scavenger receptors. Mice infused with lysophosphatidylcholine showed impairment of apoptotic clearance. This can cause a chain reaction in which the macrophage undergoes apoptosis, causing more MP shedding. If the thrombus is disrupted, the arsenal of MP's and procoagulant molecules can be released (Morel et al 2006).

During the course of thrombus buildup, PMP's may modulate angiogenesis, causing the thrombus to be vulnerable. While productive in ischemic muscle, it can prove fatal and noxious in the thrombus (Virmani et al 2005). The PMP may cause angiogenesis whereas the EMP may enhance oxidative stress leading to cell destruction. The LMP could hinder endothelial NO synthesis, leading to endothelial apoptosis.

Tissue factor was believed to have been the major initiator for a thrombus. A study involving reciprocal bone-marrow transplants between mice showed that while vessel-wall tissue factor initiates the thrombus, blood-borne tissue factor, spread by microparticles is responsible for the propagation. They reasoned that vessel-wall tissue factor is quickly covered by the plaque, and is unable to reach circulating clotting factor. PMP's and LMP's are able to circulate, alerting and gathering the needed factors (Chou et al 2004).

Artheroscelrosis

Artherosclerosis is the disease of the blood vessels by deposits of fatty plaques on vessel walls. A low level of high density lippoprotien (HDL) in the blood is a predictive factor in coronary artery disease. It is believed that the HDL is able to carry excess cholesterol and transport it to the liver. In addition,

HDL's promote endothelial cell growth and suppress apoptosis. When HDL levels are low, the endothelial cell is no longer protected and apoptosis occurs, releasing pro-coagulant microparticles (Nofer et al 2001).

Early and constant endothelial dysfunction is a promoter of artheroscelrosis. The main contributor to the buildup of plaques is MP's carrying tissue factor. Doctors generally treat this problem with percutaneous coronary intervention, or more commonly known as the angioplasty. This procedure has shown to lead to procoagulant MP release. The now circulating MP can trigger more PMP release and MP shedding. Circulating MP's carry oxidized pohsholipids on their surface, which in turn express adhesion molecules. This triggers the release of chemokines by endothelial cells. Studies have shown that circulating PMP's reached a peak 8 hours after an angioplasty parallel to the decrease in platelet count (Tramontano et al 2004). An in vitro study has shown that EMP's can induce the release of matrix metalloproteinase -2 and -9. These enzymes degrade the matrix surrounding them, contributing to endothelial dysfunction (Taraboletti et al 2002).

It has been shown that LMP levels are higher in cardiovascular and artheroscleric patients as opposed to other diseases. However, the author notates the reason for this disparity in observations may be due to different methods used to conduct the experiment. For example, different methods of counting the MP's were used, with different centrifuge steps. Furthermore, the criteria for each patient pool differed significantly (Helal et al 2011). Further testing must be done before reaching a decision.

The MPs remain circulating in the myocardial vasostructure, increasing the chances for more plaques to develop. MPs can limit myocardial perfusion through many different pathways. The NO synthase pathway is involved in angiogenesis and neural development through cellular signaling. In addition, the NO is a vasodilator as opposed to its agonist, cytokines (tumor necrosis factor) which are vasoconstrictors (Cody et al 1992).The NO synthase is produced by endothelial cells. The MP's impair the regular endothelial function, disrupting NO synthase and promoting production of cytokines. This causes vasoconstriction in the blood vessel, limiting the blood flow. PMP's can control vascular tone, as shown in an experiment involving rabbits' aortic tone (Pfister 2004).

Pulmonary Hypertension

Pulmonary hypertension is associated with a pulmonary arterial pressure greater than 25 mm Hg. It is caused by severe obstruction in the pulmonary vessel wall, thrombosis, and vasoconstriction. It has been shown that the endothelial cell is changed during pulmonary hypertension. The cell is activated causing many cell factors to be released, among them MP's. Patients suffering from pulmonary hypertension will have a higher number of circulating MP's. The circulating MP's will release many coagulant factors, such as cytokines which induce thrombo-embolic conditions (Diehl et al 2011). The increased numbers may correlate to the structural damage in the vessel wall (Amabile et al 2008).In addition, PMP's may induce smooth muscle proliferation in the vessel wall and intima thickening, both contributing to pulmonary hypertension progression (Diehl et al 2011).

Scientists had believed that EMP levels have a direct effect on pulmonary arterial pressure as shown when measured by echocardiogram. However, it has since been proven that the echocardiogram severely limits the results: therefore it is suggested to use the catheter to better test for severity of arterial pressure. In a study involving 16 patients, scientists have shown that EMP's can be both a marker of dysfunction and a contributor to impairment of vascular function in pulmonary hypertension.

EMP's can interact with the endothelium and restrict NO synthesis. By doing so, EMP's act as a paracrine factor Inhibiting NO dependent vasodilation, leading to restriction in the blood flow when needed (Cody et al 1992). The disease will progress with more changes made to the arterial wall as a result. Furthermore, EMP release may have been caused by high-sensitivity C-reactive protein and LMP's showing that MP's have many different sources and reasons for release (Amabile et al 2008) Hypertriglyceridemia enhances the production of tumor-necrosis factor and C-reactive protein, molecules shown to affect EMP release (Ferrereira et al 2004).

It is interesting to note that annexin-V was not seen on the MP surface. The author theorized that the lack of apoptosis observed as well as data collection method are responsible for this lack (Amabile et al 2008. In addition, LMP's are known to be responsible for cell adhesion. However, the leukocyte counts were similar between the patients and the controls. The author theorized that functional status of leukocytes and not their absolute number was altered (Diehl et al 2011). For both experiments the patient pool was too small as well as too heterogeneous, more experimentation is needed.

Chronic Renal Failure

Cardiovascular disease is the main cause of death in patients with chronic renal failure. Endothelial dysfunction often occurs in the beginning stage of kidney failure. Blood pressure rises as glomerular filtration rate drops. However, Faure et al (2005) have found that there is no correlation between blood pressure, glomerular filtration and MP's. Scientists used an ultrasound to measure risk assessment. The study measured MP levels before and after dialysis. Results show that compared to controls, patients suffering from kidney disease had higher MP levels. Additionally, the study showed that uremic toxins had a positive correlation with the release of EMP's. Uremia affects the vascular structure in arterial stiffness and early stages of atheromatous disease (Dursan et al 2009).

Scientists injected frogs with uremic toxins from dialysis patients. Results showed that the frog's blood vessels were eight times more permeable after being injected by uremic toxins. An abstract was published in which (Adamson 1990) said permeability occurred with uremic toxins from healthy patients, but the authors were unable to reproduce the result. They believe that results would be considerably less in humans.

However, the situation can become a risk factor if the vessel wall becomes permeable to LDL's and albumin (Harper et al 2002). In addition, there was a 37% increase in EMP release in response to the release of indoxyl sulfate (a molecule in uraemic acid). Renal failure patients show excessive formation of MP, not inclusive to EMP. EMP and LMP levels remained constant before and after the dialysis session. However, PMP count was enhanced after the session. This may be as a result of mechanical stress on the platelets which stimulates MP shedding.

The uremic state of renal failure patients can cause endothelial dysfunction on its own. However, the MP's amplify the endothelial dysfunction already in place, such as the tissue factor and NO pathways along with vasorelaxation. Renal failure patients are dependent on the endothelium for a vasodilation response (NO synthase) and suffer decreased response to cytokines. Analysis has shown that patients without a history of vascular disease before suffering from renal failure have similar levels of MP's to those who had a past history.

P-cresol, a molecule known to induce endothelial dysfunction, which impairs endothelial barrier function and response to inflammatory cytokines, was induced in vitro to an endothelial cell culture. The p-cresol promoted the formation of MP's in the cells. It affected the endothelial cell skeleton on the Rho kinase pathway. Because the cytoskeleton is important to MP release, the author theorized that MP release was induced through this pathway, not as believed before by the apoptotic process. This is specific only for renal failure patients. Further evidence shows that endothelial activation is a response to an accumulation of many toxins, not only p-cresol and indoxyl sulfate. Evidence has shown uremia to be a risk factor for diabetes, however further research must be done (Faure et al 2005).

Obesity and Metabolic Syndrome

Obesity is an increasing problem among children and adults. It has been shown that obesity correlates to increased levels of clotting factors and increased coaguable state. Oxidative stress may affect the release of MPs. Obesity is a chronic state of oxidative stress and inflammation. Obesity is one of many factors involved in metabolic syndrome. Patients suffering from metabolic syndrome are commonly susceptible to endothelial dysfunction, triggering platelet aggregation with further risk in venous thrombus formation. In addition, patients are prone to resistance to insulin, low glucose tolerance, dyslipidemia, and hypertension (Nieuwdorp et al., 2005). Metabolic syndrome patients are also at increased risk for type 2 diabetes, cardiovascular disease, and mortality (Helal et al 2011).

In a study involving obese children, it was discovered that thrombin generation is increased. Obese children have a shorter lag time (exposure) and time to peak height (highest number of MPs), higher peak height, and higher thrombin potential. The study further revealed that obese children have a shorter MP release time, allowing the coagulation pathway to begin at a faster rate. The author hypothesized that the prothrombic state is founded in obesity. He furthers this hypothesis by stating that prothrombic state will accelerate to cardiovascular disease. Helal et al (2011) show that obesity causes a chronic state of oxidative stress and inflammation, leading to cardiovascular disease. In order to prevent cardiovascular disease, control of obesity should be done at an early stage. One theory suggests that the fat depots found in obese patients lead to MP overproduction. Furthering this line of thought, it was discovered that a high-fat meal induces the release of MP's. However, a fasting blood test, even in the metabolic syndrome patient did not correlate with the controls in other studies, showing that the high-fat meal affected the blood test more than metabolic syndrome in comparison between the patients and controls. However, the study only involved 18 subjects over a space of 2 days and more research must be done (Ferreria et al 2004).

Siklar et al (2011) found that insulin and glucose levels did not affect the MP levels in the patients. This may be as a result of the use of obese children as opposed to other findings in which they used overweight children and adults with type 2 diabetes. A study involving 88 metabolic syndrome patients not currently suffering from any other diseases or syndromes, tested for MP levels. It was found that Annexin-V, PMP, EMP, and LMP levels were significantly higher than controls. The hypothesis is that platelet, erythrocyte, and endothelial derived MP's were released in a general response and the monocyte derived MP's are released by more severe conditions, such as cardiovascular disease (Helal et al 2011). Arteaga et al (2006) conducted a study involving 33 patients with no previous history. He found that only the EMP levels were raised in the metabolic syndrome patient.

Biomarker

As previously stated, many diseases have higher levels of MP when compared to controls. In order to use microparticles as a tool, further studies must be done to accurately count the MP levels in the blood. As of now, methods are not completely developed (Kornek and Schuppan 2012). Current ways to count MP are: flow cytometry, fluorescent monoclonal antibodies, and two step differential centrifugation. Flow cytometry depends on membrane-specific antigens. The blood sample is incubated with an antibody for 30 minutes. At the end of the prescribed time, the sample is diluted and count beads are added to each sample according to internal standard (Faure et al 2006). A recent innovation adds different colors to the beads, allowing different phenotypes to be easily differentiated (Tramontano et al 2004). The sample is then counted by the flow cytometer. MP's are defined by their sum (>1μm) (Faure et al 2006).

Fluorescent monoclonal antibody count depends on antigen and antibody interactions. The specific antibody is labeled with a fluorescein isothiocyanate. The sample is then triggered by signals, and results are charted (Arteaga et al 2006). The final method is centrifugation. Most studies used a two-step centrifugation to get rid of cells that were previously treated. Time length and centrifuge speed differ based on authors preferences. The sample is then centrifuged a second

time at a faster speed and longer period of time in a microcentrifuge to isolate the MP pellet. The isolated MP pellet is then suspended in a buffered saline at one-tenth the original volume. The MP is then counted by flow cytometer (Ulal and Pitsetsky 2010). The different methods may be mixed and matched, as done by Helalet al(2011). The study used centrifugation, flurochrome technology, and flow cytometry to attempt to achieve as accurate a number as possible.

Pharmacology

Inflammation is a significant contributor to endothelial dysfunction and subsequently, artheroscelrosis. MP's promote endothelial dysfunction with the help of pro-inflammatory cellular adhesion molecules triggered by tumor necrosis factor. Tumor necrosis factor also activates the Rho kinase pathway. Statins are commonly used to treat artherosclerosis. They are known for their anti-inflammatory effects and LDL inhibition. Fluvastatin has been shown to reduce the synthesis of cellular adhesion molecules as well as inhibit Rho activation. While the exact mechanism is not yet understood, it is believed that Rho activation plays an important role in EMP release. In addition, Rho kinase is involved in cytoskeleton organization. By suppressing Rho kinase activation, actin cytoskeleton organization is also inhibited, leading to EMP inhibition as well (Tramontano et al., 2004). Another statin, Pravastatin has been shown to reduce the fibrinogen receptor on MPs in diabetes type 2 patients. Fibrinogen is an important aspect in thrombus growth. With repression of the receptor, thrombus growth can be lessened and cardiovascular events can be reduced (Baron et al 2012).

Conversely, in a study involving patients with chronic renal failure, statins did not show any decrease in MP levels when compared to controls. This may be a result of other factors in the patients, such as uremia levels (Faure et al 2006)

Oxidative stress has been shown to affect cardiovascular disease. Low levels of antioxidants (vitamin c) have been associated with inflammation and severity of disease. Vasospasms and neonintimal thickening after ballooning were improved after being treated with antioxidants. Morel et al (2003) conducted a placebo trial study with 61 myocardial infarction patients. Half of the group was placed on a vitamin C supplement for 5 days while others were given a placebo. Results were modest, a 10% reduction in overall MP count. However, the placebo group suffered an up to 44% increase in MP levels. It is believed that antioxidants prevent the ongoing process of MP shedding. This inhibition result was not observed in low-risk patients, perhaps due to the different nature of oxidative stress in the low-risk as opposed to high-risk patients. Additionally, vitamin C reduced cell apoptosis, with up to 70% decrease of EMP in diabetic patients. An early treatment of antioxidants can reduce redox reactions, leading to a decrease of cardiovascular disease (Morel et al 2003).

MP's can also be used as a treatment for bleeding disorders. In a trial run by Hrachovinova et al (2003), hemophilic mice were infused with P-selectins and immunoglobins. This procedure was done in vitro as well. This in turn initiated the release of tissue-factor containing MP's, causing coagulation to occur. Fibrin formation occurred at a faster rate after incubation with p-selectin and immunoglobins. This shows that hemophiliacs may have other treatments available to help in thrombus formation.

Conclusion

Microparticles have come a long way since discovered in 1969. They have been shown to be involved in many pathways of the human body, such as NO synthase and Rho kinase. Their main contribution lies in thrombus formation, helping the body recover from injury. However, in case of metabolic disturbance, the MP may become a pathogen to the patient. It will inhibit pathways needed and contribute to cardiovascular disease. Studies are currently being done to examine the exact pathway and correct the problem. As of now, certain medications have been discovered to help control MP levels in the blood. In addition, scientists are considering using MP levels as a biomarker. The MP levels can become a useful tool to predict cardiovascular and other diseases. MP is also emerging as a healing tool for hemophiliacs and other bleeding disorders.

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