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IMPORTANCE OF PLATELET AGGREGATION IN PATIENTS WITH END-STAGE RENAL DISEASE

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SUMMARY – The exact etiology of the conflicting hemostatic disorder in the advanced stage of chronic renal disease, i.e. prothrombotic *versus* bleeding tendency, is not completely understood. Abnormal platelet function in patients with renal failure is not caused by high concentrations of urea, although the presence of fibrinogen fragments may prevent binding of normal fibrinogen and formation of platelet aggregates. Hemostatic abnormalities in end-stage kidney disease may be affected, to some extent, by the choice of renal replacement therapy. Patients on hemodialysis have an increased risk of thrombotic events, primarily due to the release of thromboxane A2 and adenosine diphosphate into the circulation, as well as platelet degranulation. Some activation of platelets occurs due to the exposure of blood to the roller pump segment, but microbubbles may also play a role. Renal transplantation is the treatment of choice for patients with end-stage renal disease. Immunosuppressive therapy is associated with an increased risk of thromboembolic complications. Additional research is required to identify the potential benefits of different immunosuppressive therapies in relation to platelet aggregation, keeping in mind the long term need for immunosuppression in renal transplant patients.

Key words: *Platelet aggregation; Kidney failure, chronic; Renal dialysis; Kidney transplantation; Immunosuppressive agents*

Introduction

The high mortality rate associated with chronic kidney disease (CKD) is mainly due to the increased incidence of cardiovascular disease, which contributes to more than half of deaths in patients with end-stage renal disease (ESRD). Thrombosis is a common complication of ESRD, particularly in patients on hemodialysis. Platelet dysfunction, which leads to an increased risk of bleeding tendency, also plays an important role. Thrombotic complications are thought to be a net outcome of both underlying kidney disease, complicated by renal failure, and its management through dialysis or renal transplantation.

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In the present paper, we discuss the problems associated with platelet aggregation in ESRD patients and renal transplant recipients.

Platelet Aggregation – Normal Clotting Process

Hemostasis is the process of blood clot formation that maintains the integrity of the circulatory system after vascular damage. There are two different and independent pathways that can activate thrombus formation after the injury:

- one is exposure of the subendothelial collagen initiating platelet activation, and
- the other is initiated by tissue factor derived from blood vessel wall or present in flowing blood, resulting in thrombin formation[1].

Clot formation requires platelet activation and generation of thrombin and fibrin in good balance with

plasmin-induced clot lysis[2]. The first step after vascular injury is exposure of subendothelial elements such as collagen and laminin. Platelet glycoprotein receptor GP-IV binds collagen and mediates platelet adhesion and activation at the site of injury. Other glycoprotein receptors GP Ib-V-IX interact with collagen-bound von Willebrand's factor (vWF), which is also required for platelet adhesion.

At the same time, the independent pathway of blood coagulation and platelet activation is initiated by tissue factor (TF) released into the circulation after vascular damage. Platelet activation initiated by this pathway does not require disruption of the endothelium and is independent of vWF and glycoprotein VI. Tissue factor binds to the active factor VIIa forming the TF/FVIIa complex initiating a proteolytic cascade.

The final goal is to generate thrombin, which converts fibrinogen to fibrin, but also activates platelets by binding to its receptor (protease-activator receptor-1), and results in the release of adenosine diphosphate (ADP), serotonin and thromboxane A2. These are platelet agonists, which activate other platelets and amplify the signal for thrombus formation. The release of ADP stimulates platelet activation through two ADP receptors, P2Y1 and P2Y12 (antiplatelet drugs directed against these receptors). Thromboxane A2 is synthesized in the platelets and functions as platelet agonist and vasoconstrictor[1].

Clot Lysis – Regulatory Mechanism

The vessel wall with its inner lining of endothelium contains thromboregulators, such as nitric oxide (NO), prostacyclin and ectonucleotidase, which together provide defense against thrombus formation.

Prostacyclin is synthesized in the endothelium by COX2 and inhibits platelet aggregation and thromboxane A2 mediated vasoconstriction.

Nitric oxide is produced by endothelial cells and inhibits platelet adhesion and aggregation[2]. The most important inhibitors of the clot formation are antithrombin, tissue factor pathway inhibitor (TFPI), protein C and protein S. These inhibitors act in different ways: antithrombin neutralizes enzymes in the coagulation cascade (II, IX, X, XII); TFPI inhibits factor X and complex TF/FVIIa; and protein C and protein S inactivate FVa and FVIIIa.

The most important component of the fibrinolytic system is the proteolytic enzyme plasmin, which cleaves the fibrin degradation products. Its precursor plasminogen is synthesized in the liver and can be activated by binding to fibrin or tissue plasminogen activator (tPA) derived from vascular endothelium. The activity of plasmin is regulated by the endothelium, which secretes tPA and plasminogen activator inhibitors (PAI-1 and PAI-2)[1].

Disorder of Platelet Aggregation in End-Stage Renal Disease

Chronic renal failure is characterized by a progressive and irreversible decline in renal function over the course of at least 3-6 months. Full manifestations of the syndrome are seen when the glomerular filtration rate (GFR) decreases below 25 mL/min. Patients with clearance below 10 mL/min (ESRD) are dialysis dependent until successful transplantation. Dialysis may take the form of intermittent hemodialysis using arteriovenous fistula or central venous catheter, or continuous ambulatory peritoneal dialysis (CAPD) *via* an implanted catheter[3].

In the advanced stage of chronic renal disease, patients suffer from procoagulant abnormalities (impaired release of tPA, increased PAI-1, elevated fibrinogen and D-dimer, and increased TF/FVIIa complex) leading to excessive cardiovascular events, as well as from platelet dysfunction, leading to an increased risk of cutaneous, mucosal or serosal bleeding tendency[2]. Platelet dysfunctions are observed mainly in advanced uremia before starting dialysis treatment[4].

Several factors contribute to platelet dysfunction in patients with ESRD, such as impaired function of platelet glycoproteins like GPIIb/IIIa and altered release of ADP and serotonin from platelet granules, which all lead to impaired platelet adhesion and aggregation. Certain uremic toxins such as guanidinosuccinic acid and methyl guanidine may contribute to platelet dysfunction by stimulating NO release from the endothelium, which again inhibits platelet adhesion and aggregation[2].

It appears that abnormalities in both the platelets and the plasma play a role in subnormal platelet aggregation in uremic state. Urea and other nitrogenous compounds such as guanidinosuccinic acid, phenols, and a group of substances called uremic middle mole-

cules have been incriminated in the decreased platelet aggregation. However, analysis of a rare syndrome of familial azotemia, characterized by high plasma urea resulting from impaired urinary excretion but normal renal function, failed to found any abnormality in platelet function. It was concluded that abnormal platelet function in patients with renal failure is not caused by high concentrations of urea[5].

In the normal clotting process, fibrinogen binds to the glycoprotein receptor IIb/IIIa complex on the activated platelet membrane and creates a bridge between adjacent platelets. As a result of multiple interactions of this type, platelet aggregates are formed. Uremia is characterized by the presence of fibrinogen fragments, which are not present in normal plasma. These fragments bind to some glycoprotein IIb/IIIa sites on the platelet membrane, preventing the binding of normal fibrinogen and forming of platelet aggregates[6].

The importance of circulating toxins is based on the observed beneficial effect of acute dialysis on platelet dysfunction[4].

Platelet Aggregation in Hemodialysis Patients and Patients on Continuous Ambulatory Peritoneal Dialysis (CAPD)

Patients on hemodialysis (HD) have an increased risk of thrombotic events[7]. The most common thrombotic complication in hemodialyzed patients is thrombosis of vascular access, which is a major cause of hemodialysis-associated morbidity, while cardiovascular and cerebrovascular incidents are major causes of dialysis-associated mortality.

Several studies have pointed to inflammation as an important contributor to thrombotic complications in hemodialyzed patients. Indeed, ESRD is currently perceived as a state of chronic or recurrent inflammation. Furthermore, the inflammatory response is exacerbated in hemodialyzed patients as a result of bioincompatibility of artificial materials and dialysis water. Elevated levels of inflammatory mediators and acute-phase reactants, such as tumor necrosis factor (TNF), interleukin-6 (IL-6), C-reactive protein (CRP), and fibrinogen have been repeatedly demonstrated in patients on dialysis. Another contributor to dialysis-associated inflammation is complement[8].

A comparative study on hemostasis in CAPD and HD patients observed platelet dysfunction in both groups of dialyzed patients that was probably related to uremic toxins present in the circulation. It was also observed that ristocetin-induced platelet aggregation was significantly higher in CAPD compared to HD patients.

Hemodialysis with extracorporeal circulation and exposure to heparin may contribute to the prothrombotic state due to recurrent platelet stimulation leading to their hyperaggregability, reduced levels of heparin cofactor II, and increased concentrations of coagulation factors. Patients on CAPD showed evidence of a higher degree of hypercoagulation than HD patients. Thus, hemostatic abnormalities in ESRD may be affected to some extent by the choice of renal replacement therapy[9].

According to the results of the study that correlated platelet dysfunction and plasminogen levels in HD patients, platelet aggregation positively correlated with plasminogen levels, which were significantly lower in HD patients[10].

Substantial activation of platelets may occur during the course of HD. Platelet surface markers show evidence of platelet degranulation. Some activation occurs due to exposure of blood to the roller pump segment, and micro bubbles may also play a role[11].

Results of the investigation of prothrombotic tendency in CAPD patients showed that the main reason for the prothrombotic state in these patients was chronic activation of coagulation system and elevated levels of fibrinogen. The fibrinolytic system and platelets did not contribute to this prothrombotic tendency[12].

Platelet Aggregation in Renal Transplant Patients

The treatment of choice in ESRD is kidney transplantation, as it improves survival and quality of life. However, the shortage of available donors combined with contraindications to transplantation in some patients results in the necessity of sustaining these persons on dialysis for a variable period of time[8]. Improvements in immunosuppressive therapy have reduced early allograft loss due to acute rejection to very low levels. Early allograft loss due to acute thrombotic complications remains a constant and proportionally increasing complication of renal transplantation[13].

Most people who have a kidney transplant need to take at least one immunosuppressive drug for the

rest of their lives. Rejection of a kidney is most likely to happen within the first 3 months after transplantation. During this time, people often take a combination of three or four different drugs, which is called initial treatment:

- induction with polyclonal or monoclonal antibodies,
- calcineurin inhibitors (cyclosporine or tacrolimus),
- antiproliferative agents (such as azathioprine or mycophenolate), and
- corticosteroids.

Maintenance immunosuppressive therapy is administered to almost all renal transplant recipients to help prevent acute rejection and loss of renal allograft. Although an adequate level of immunosuppression is required to damp the immune response to the allograft, the level of chronic immunosuppression is slowly decreased over time (as the risk of acute rejection decreases).

The optimal maintenance immunosuppressive therapy in renal transplantation is not established. The major immunosuppressive agents that are currently used in various combination regimens are corticosteroids (primarily oral prednisone), azathioprine, mycophenolate mofetil (MMF), mycophenolate sodium, cyclosporine (in standard form or microemulsion), tacrolimus, everolimus, and rapamycin (sirolimus)[14].

Immunosuppressants

Immunosuppressive agents are used for induction (intense immunosuppression in the initial days after transplantation), maintenance, and reversal of established rejection[14]. Azathioprine, which is derived from 6-mercaptopurine, was the first immunosuppressive agent to achieve widespread use in organ transplantation. Upon the introduction of cyclosporine, azathioprine has become a second-line drug[14].

Calcineurin inhibitors

Cyclosporine, a cornerstone of immunosuppression in transplantation, is a prodrug that engages cyclophilin, an intracellular protein of the immunophilin family, forming a complex that then engages calcineurin. The adverse effects of cyclosporine, which are related to the concentration of the drug, include nephrotoxicity, hypertension, hyperlipidemia, gingival hyperplasia, hirsutism and tremor. Cyclosporine can also induce the hemolytic-uremic syndrome and post-transplantation diabetes mellitus. Recent developments include monitoring of the peak cyclosporine levels two hours after administration to better reflect exposure to the drug.

Tacrolimus engages another immunophilin (FKBP12) to create a complex that inhibits calcineurin with greater molar potency than does cyclosporine. Tacrolimus resembles cyclosporine in that it can result in nephrotoxicity and the hemolytic-uremic syndrome, but it is less likely to cause hyperlipidemia, hypertension and cosmetic problems, and more likely to induce post-transplantation diabetes[14].

Inosine monophosphate dehydrogenase inhibitors

Mycophenolic acid inhibits inosine monophosphate dehydrogenase, a key enzyme in purine synthesis. Mycophenolate mofetil is a prodrug that releases mycophenolic acid, and in large-scale trials with cyclosporine, it was superior to azathioprine in preventing rejection of kidney transplants[14].

Target-of-rapamycin inhibitors

Sirolimus and everolimus engage FKBP12 to create complexes that engage and inhibit the target of rapamycin but cannot inhibit calcineurin. The principal nonimmune toxic effects of sirolimus and everolimus include hyperlipidemia, thrombocytopenia, and impaired wound healing. Sirolimus and everolimus may reduce cytomegalovirus disease[14].

Immunosuppressive Therapy – Platelet Aggregation

Immunosuppressive therapy including cyclosporin A is associated with an increased risk of thromboembolic complications. It has been reported that cyclosporin A enhances platelet aggregation *in vitro* and subsequent thromboxane A2 release, and reduces prostacyclin production by the vessel wall[15].

Cyclosporine impairs endothelium dependent vasorelaxation and decreases prostacyclin (PGI2) release from vascular rings and cultured human endothelium. PGI2 is synthesized predominantly by cyclooxygenase 2 (COX2) in healthy humans. Cyclosporine, but not tacrolimus and rapamycin, inhibits COX2 in human vasculature smooth muscle. Rapamycin and tacrolimus

may actively suppress platelet and renal thromboxane formation. Platelet aggregation, thromboxane formation by activated platelets and renal thromboxane excretion are lower in patients treated with tacrolimus and rapamycin as compared with cyclosporine[16].

According to the results of a study comparing the effects of immunosuppressive drugs on platelet aggregation in renal transplant patients, cyclosporine-treated renal transplant patients showed enhanced platelet activation compared with tacrolimus group[17].

Platelet activity is enhanced in hypercholesterolemia, which is commonly observed in renal transplant patients. This could be a crucial factor in the pathogenesis of atherosclerotic lesion formation and cardiovascular events. Activated platelets tend to aggregate and are found close to atherosclerotic plaques. The membrane protein P-selectin, which is released from endothelial cells and activated platelets, binds leukocytes to endothelium and mediates the rosetting of activated platelets around neutrophils and monocytes, which could be important in the development of atherosclerosis[15].

Conclusion

In the advanced stage of chronic renal disease, patients suffer from procoagulant abnormalities leading to excessive cardiovascular events, as well as platelet dysfunction, manifested as an increased risk of bleeding tendency. The exact etiology of this conflicting hemostatic disorder, i.e. prothrombotic *versus* bleeding tendency, is not completely understood. Hemostatic abnormalities in ESRD may be affected, to some extent, by the choice of renal replacement therapy (HD *vs*. CAPD). The treatment of choice in ESRD is renal transplantation, which requires permanent need of immunosuppression. Immunosuppressive therapy is associated with an increased risk of thromboembolic complications. It has been reported that cyclosporin A, a cornerstone of immunosuppression in transplantation, enhances *in vitro* platelet aggregation and subsequent thromboxane A2 release, and reduces prostacyclin production by the vessel wall. Tacrolimus and rapamycin influence platelet aggregation and thromboxane formation to lesser extent as compared to cyclosporine. Additional research is required to identify the potential benefits of different immunosuppressive drugs in relation to platelet dysfunction, keeping in mind the long-term need of immunosuppression in renal transplant patients.

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Sažetak

ZNAČENJE AGREGACIJE TROMBOCITA U BOLESNIKA S BUBREŽNIM ZATAJENJEM

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Točna etiologija proturječnih hemostatskih poremećaja u terminalnom stadiju bubrežne bolesti, tj. tromboze i sklonosti krvarenju, nije u potpunosti razjašnjena. Poremećena funkcija trombocita u bolesnika s bubrežnim zatajenjem nije uzrokovana povišenom koncentracijom ureje, premda prisutnost fragmenata fibrinogena može spriječiti vezivanje normalnog fibrinogena, odnosno stvaranje agregata tromobocita. Na poremećaj hemostaze kod bolesnika s bubrežnim zatajenjem može utjecati i izbor nadomjesnog bubrežnog liječenja. Bolesnici na hemodijalizi imaju povećani rizik tromboze prvenstveno zbog oslobađanja tromboksana A2 i ADP-a u cirkulaciju, kao i zbog degranulacije trombocita. U stanovitoj mjeri trombociti se aktiviraju i prolaskom krvi kroz sustav crpki, dok moguću ulogu imaju i mikromjehurići. Transplantacija bubrega je metoda izbora u liječenju bolesnika s bubrežnim zatajenjem. Imunosupresivna terapija je povezana s povećanim rizikom razvoja trombembolijskih komplikacija. Imajući u vidu dugotrajnu potrebu za imunosupresivnim liječenjem kod bolesnika s transplantiranim bubregom potrebna su daljnja istraživanja radi utvrđivanja mogućeg povoljnog učinka različitih imunosupresiva u odnosu na agregaciju trombocita.

Ključne riječi: *Trombociti, agregacija; Bubrežno zatajenje, kronično; Bubreg, transplantacija; Imunosupresivi*