Alternatives to platelet transfusions in the management of platelet dysfunction or thrombocytopenia

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SUMMARY

Alternatives to platelet transfusions are important in preventing the adverse events associated with platelet transfusions. The use of platelet alternatives has not been extensively compared with platelet transfusions, although studies suggest that bleeding can be controlled and transfusions prevented. A variety of pharmacologic agents are available, which generally work by augmenting different hemostatic mechanisms. In addition to pharmacologic agents, recombinant activated factor VII is available for controlling inherited qualitative platelet disorders such as Glanzmann's thrombasthenia. For the treatment of chemotherapy-induced thrombocytopenia, the use of growth factors may shorten the duration of thrombocytopenia and prove to be an approach for reducing or preventing platelet transfusions. Although interesting research has been carried out for several decades in the aim of developing a platelet substitute, it is unlikely that a platelet substitute will be available for clinical use in the near future.

INTRODUCTION

There is keen and justifiable interest in alternatives to platelets and in platelet substitutes. However, when the hemostatic function of platelets is absolutely needed there is no substitute or alternative to a platelet transfusion at the present time. In fact there have not been any controlled studies comparing platelet transfusions to the alternatives that will be discussed here. Nonetheless, several small studies report that a variety of pharmacologic approaches to control bleeding may be used in well-defined situations where platelet dysfunction is present. For severe thrombocytopenia, a few promising approaches are being studied. These include growth factors to stimulate thrombopoiesis and recombinant activated factor VII (rFVIIa).

This paper will review non-transfusion strategies for managing thrombocytopenia and platelet dysfunction. This paper will also review the different approaches being considered for the development of platelet substitutes, but the reader should be aware that much more research is needed in this area before one can expect the clinical application of platelet surrogates. It is important to note that in the management of thrombocytopenia the best strategy in correcting the resultant bleeding is to define the cause of the

thrombocytopenia and, where possible, to reverse or remove the causative agent or disease process. Where this is not possible, the approaches discussed below are applicable.

ANTIFIBRINOLYTIC AGENTS

Two synthetic derivatives of the amino acid lysine, 6-aminohexanoic acid (epsilon-aminocaproic acid) and 4-(aminomethyl)cyclohexanecarboxylic acid (tranexamic acid), have antifibrinolytic activity in humans.[1] Both drugs bind reversibly to plasminogen and thereby block the binding of plasminogen to fibrin and its activation and transformation to plasmin. Epsilon-aminocaproic acid and tranexamic acid (which is about 10 times more potent than epsilon-aminocaproic acid and has a longer half-life) are effective even when bleeding is not associated with laboratory signs of excessive fibrinolysis.[2] Since both drugs enter the extravascular space and accumulate in tissues, the basis for their efficacy is thought to be the inhibition of tissue fibrinolysis and the consequent stabilization of clots.

Clinical application of antifibrinolytic agents in platelet disorders

Antifibrinolytic agents have proven useful in women with excessive menstrual bleeding caused by inherited qualitative platelet disorders or von Willebrand's disease. Tranexamic acid reduces blood loss by 40–50%, as documented in a randomized controlled trial in 76 women.[3] The drug is thought to act by inhibiting plasminogen activator, which is present in high concentrations in the endometrium. Its use is recommended only when the presence of organic lesions in the uterus has been ruled out and when combined estrogen-progestogen preparations, which control dysmenorrhea and menstrual irregularity more effectively, are unacceptable or contraindicated. The recommended oral dose of tranexamic acid is 10–15 mg per kg of body weight every 8 hours.

Patients with prolonged periods of megakaryocytic thrombocytopenia secondary to chemotherapy, myelofibrosis, leukemia, myelodysplastic syndromes, and aplastic anemia have been treated with epsilon-aminocaproic acid for prophylaxis and control of bleeding. In one uncontrolled study, bleeding that was primarily of oral mucosal origin was stopped in 14 of 15 thrombocytopenic patients who received epsilon-aminocaproic acid at a dose of 4 g every 6 hours during an acute bleeding episode, followed by 3–4 g every 8 hours after hemostasis was achieved. Platelet counts were unaffected, yet transfusion requirements in four patients who received long-term epsilon-aminocaproic acid were reduced from an average of 95 units of platelets per month to 4 units per month.[4]

Side effects of antifibrinolytic agents

The side effects of tranexamic acid and epsilon-aminocaproic acid are dose-dependent and usually involve the gastrointestinal tract (nausea, vomiting, abdominal pain, and diarrhea). The main risk associated with these drugs is that thrombotic complications will result from the inhibition of fibrinolysis, which is a natural mechanism of defense against the formation of thrombus. Myopathy and myonecrosis are rare complications associated with long-term use of high-dose epsilon-aminocaproic acid. Baseline and serial muscle enzymes should be monitored if patients are receiving long-term therapy.[2–4]

DESMOPRESSIN

Plasma concentrations of factor VIII, the clotting factor that is deficient or defective in patients with hemophilia A, and von Willebrand factor, the adhesive protein that is deficient or defective in patients with von Willebrand's disease, can be increased for a short time by the administration of 1-deamino-8-d-arginine vasopressin (desmopressin, or DDAVP), an analogue of arginine vasopressin.[5] These effects, which mimic replacement therapy with blood products, form the rationale for the use of desmopressin in the treatment of patients with hemophilia A or von Willebrand's disease.[5,6]

Desmopressin has also been used in patients with other congenital and acquired platelet disorders.[6–8] In such patients, the effect of desmopressin may be mediated by the attainment of supranormal plasma concentrations of von Willebrand factor and the appearance of ultra-large multimers of this factor,[5] which support platelet adhesion to the vascular subendothelium more actively than multimers of normal size. Increased hemostasis may also be mediated by high plasma concentrations of factor VIII, a rate-accelerating factor in the process of fibrin formation.

Early investigators documented the efficacy of desmopressin in patients who had prolongation of the bleeding time but no evidence of von Willebrand's disease. Platelet studies demonstrated one of the following patterns: normal or abnormal response to aggregating agents or a storage-pool defect. Those patients with a prolonged bleeding time and normal aggregation studies were classified as having an isolated prolongation of the bleeding time.[8] Six additional reports have confirmed the efficacy of desmopressin in these groups of patients. Two studies have examined the effectiveness of desmopressin in patients on aspirin therapy. In a controlled trial, Mannucci *et al.* found that desmopressin significantly shortened the bleeding time in six healthy volunteers who had ingested a single 500-mg dose of aspirin. However, the bleeding time in these volunteers was only minimally elevated after aspirin ingestion. In another study, desmopressin corrected the prolonged bleeding time of two aspirin-treated patients who then underwent invasive procedures without excessive blood loss. Additional studies are needed to define the role for desmopressin in this clinical setting.[9–11]

Desmopressin administration

The optimal intravenous or subcutaneous dose of desmopressin in patients with congenital bleeding disorders is 0.3 μg/kg, and the optimal intranasal dose is 300 μg in adults and 150 μg in children.[11,12] Plasma concentrations of factor VIII and von Willebrand factor are approximately doubled or quadrupled by the administration of desmopressin, reaching a peak 30–60 minutes after intravenous infusion and 60–90 minutes after subcutaneous or intranasal administration.[11,13] These doses can be repeated as clinically necessary at intervals of 12–24 hours, but tachyphylaxis may occur after three or four doses.[14]

Desmopressin is the treatment of choice for patients with mild hemophilia A or type I von Willebrand's disease who have spontaneous bleeding or who are scheduled to undergo surgery. It shortens or normalizes the bleeding time in some patients with congenital defects of platelet function. Although the effect of desmopressin on a laboratory measure such as the bleeding time may not correspond to a hemostatic effect in patients, the results in a few well-studied cases suggest that desmopressin may be an alternative to blood products during or after surgery or childbirth in such patients.

Desmopressin has also been used in patients with uremia who have complex abnormalities of hemostasis reflected in part by a prolonged bleeding time. In a group of these patients who were given an intravenous infusion of desmopressin, the prolonged bleeding time became normal for 4–6 hours in about 75%. Desmopressin given before invasive procedures (such as biopsies and major surgery) seems to prevent bleeding, but controlled studies of this effect are lacking. Currently, the clinical use of desmopressin in patients with uremia is based on the link between the degree of prolongation of the bleeding time and the patient's tendency toward excessive bleeding.[7,11,15]

Side effects of desmopressin

Common side effects include mild facial flushing and headache. Because of its potent antidiuretic effect, desmopressin can cause water retention and hyponatremia. In patients given more than one dose, plasma sodium and body weight should be measured daily and excessive administration of fluids avoided. Arterial thrombosis (sometimes causing fatal stroke or myocardial infarction) has occurred in a few patients treated with desmopressin. In patients at high risk for thrombosis (such as those undergoing coronary-artery bypass grafting), there was no excess rate of thrombotic complications among those given desmopressin.[11,16]

Desmopressin, conjugated estrogens, and anemia correction in uremia

Patients with uremia frequently experience bleeding secondary to an acquired platelet dysfunction potentiated by severe anemia. Platelets from uremic patients have multiple abnormalities, including a storage pool defect, and when studied in an *in vitro* system, they show decreased adhesiveness to human vessel subendothelium. There are reports that recently reported that the platelets contain decreased von Willebrand factor and that the high-molecular-weight forms of von Willebrand factor are relatively decreased in plasma from uremic patients. In uremia the degree of prolongation of bleeding time is the test that correlates best with the likelihood of clinical bleeding. Thus, the success of hemostatic agents in uremic patients is measured

by their effect on bleeding time as well as by their ability to induce hemostasis during surgical procedures such as renal biopsies. Although hemodialysis can induce partial correction of bleeding diathesis, hemostatic agents such as desmopressin and conjugated estrogens can be used to induce complete correction of prolonged bleeding time. Desmopressin acts within 1–2 hours for short-term control of bleeding, probably by promoting platelet adhesion through increasing the concentration of high-molecular-weight multimers of von Willebrand factor.[11,17] In a double-blind, randomized, placebo-controlled trial, 12 patients showed partial correction of prolonged bleeding time lasting at least 4 hours after the intravenous administration of desmopressin (0.3 μg/kg). Nine patients then underwent invasive procedures, including renal biopsy and nephrectomy, with no excessive bleeding. The effect of estrogen on correcting prolonged bleeding time in uremic patients was first reported by Liu *et al.* in 1984.[18] This drug may be given over a longer period of time than desmopressin and is another useful approach in the management of uremic bleeding.

Along with the use of desmopressin, increasing the hematocrit in uremic, anemic patients to 30% shortens the bleeding time, which may be explained by the observation that an elevated red cell volume increases radial migration of platelets to the vessel wall. The administration of erythropoietin to uremic patients induces a similar reduction in bleeding time by increasing the red cell mass. Moia *et al.* recommended that uremic patients who are being treated with erythropoietin should target a hematocrit of 30% as a goal of therapy, because higher values may be associated with an increased incidence of thrombotic complications.[19–21]

RECOMBINANT ACTIVATED FACTOR VII

Coagulation factor VII (FVII) is a serine protease and a vitamin K-dependent coagulation factor. The primary sequence of FVII is similar to other vitamin K-dependent coagulation proteases and it circulates in blood as a single-chain zymogen of 406 residues. Production of recombinant human activated factor VII (rFVIIa) requires a mammalian expression system. The gene for human FVII, isolated from chromosome 13, is transfected into a baby hamster kidney (BHK) cell line that secretes FVII into the culture medium in its single chain form. The product is purified with murine monoclonal anti-FVII antibodies and is subjected to treatment with 0.1% Triton X-100 to inactivate enveloped viruses. Subsequent ion exchange chromatography further purifies the product and causes auto-activation, producing an activated form of FVII.[22]

Activated factor VIIa (FVIIa) promotes hemostasis by enhancing thrombin generation through direct activation of FX after complexing with tissue factor (TF) at the site of injury. FVIIa by itself does not have proteolytic activity. In normal individuals a small amount of thrombin formed via the FVIIa/TF pathway activates FV and FVIII as well as platelets accumulated at the site of injury. The activated platelets then provide negatively charged surfaces for further thrombin generation. The administration of rFVIIa enhances thrombin generation through direct activation of factor X independent of factor VIII or factor IX, on thrombin-activated platelet surfaces, ensuring a full thrombin burst needed for formation of a fully stabilized fibrin plug. rFVIIa may also directly activate platelets at the site of injury.

rFVIIa is approved by Food and Drug Administration for the treatment of bleeding episodes in patients with hemophilia A or B, when inhibitors to these factors are present. Currently, the safety and efficacy of rFVIIa is being investigated in a number of trials designed to establish the efficacy of rFVIIa as a rescue treatment in episodes of severe life-threatening bleeding, stem cell transplantation, intracerebral hemorrhage and trauma. Several case reports in the literature support the use of rFVIIa in bleeding non-hemophiliac patients and thrombocytopenic patients, but no formal randomized trials have been presented and the drug is not licensed for these indications.[22] In Europe, rFVIIa is approved for use in alloimmunized Glanzmann's patients who are bleeding and need therapy to stop bleeding.

Antibodies to glycoprotein IIb–IIIa and/or human leukemia antigens may render platelet transfusions ineffective to stop bleeding or to cover surgery in patients with Glanzmann's thrombasthenia. Anecdotal reports suggest that rFVIIa might be a therapeutic alternative in these situations. An international survey was conducted to evaluate further the efficacy and safety of rFVIIa in Glanzmann's thrombasthenia patients. The investigators analyzed the use of rFVIIa during 34 surgical/invasive procedures and 108 bleeding episodes in 59 Glanzmann's thrombasthenia patients including 29 with current or previous antiplatelet antibodies, and 23 with a history of refractoriness to platelet transfusion.

rFVIIa was effective in 29 of the 31 evaluable procedures, and in 77 of the 103 evaluable bleeding episodes of which eight had a recurrence. A significantly higher success rate was observed in severe bleeding episodes when an arbitrarily defined 'optimal regimen' derived from the Canadian pilot study results ($\geq$80 g μ/kg rFVIIa/injection, dosing interval $\leq$2.5 h, three or more doses before failure declaration) was used compared with other regimens (77%; 24/31 *vs.* 48%, 19/40; $P = 0.01$, χ^2 test). Patients given maintenance doses had significantly fewer recurrences within 48 h of bleed cessation compared with those not given any ($P = 0.022$, Fisher's exact test). One thromboembolic event and one blood clot in the ureter occurring in surgical patients following prolonged continuous infusion of high-dose rFVIIa and antifibrinolytic drug use have been previously reported. Thus, rFVIIa seems a potential alternative to platelet transfusion in Glanzmann's thrombasthenia patients, particularly in those with antiplatelet antibodies and/or platelet refractoriness.[22]

GROWTH FACTORS

Megakaryocytic growth factors

Since the discovery of thrombopoietin, there has been much progress in the clinical development of recombinant thrombopoietins but none has yet been approved for clinical use. Two recombinant thrombopoietins have been subjected to intensive clinical investigation, first a glycosylated molecule produced in Chinese hamster ovary cells consisting of the full-length, native human amino acid sequence which has a circulatory half-life of 20–40 hours; second, a non-glycosylated, truncated molecule produced in *Escherichia coli* composed of the first 163 amino acids of the native molecule and chemically coupled to polyethylene glycol. This half of the native molecule is 50% similar to erythropoietin, contains the entire receptor-binding domain, but has a very short circulatory half-life and no biologic activity *in vivo* due to the absence of the remaining, carbohydrate-rich portion of the native molecule. In the second available formulation, the addition of the polyethylene glycol moiety serves to stabilize the molecule in the circulation and replaces the carbohydrate domain. Pegylated recombinant human megakaryocyte growth development factor (PEG-rHuMGDF) has a half-life of 30–40 hours. Clinical development of this molecule has

been stopped because of the development of antibodies, leading to thrombocytopenia, in approximately 8% of subjects.[23]

There are several new molecularly designed platelet growth factors based upon the structure of thrombopoietin or its receptor that are just entering preclinical testing. One of these, promegapoietin, is a molecular modification of thrombopoietin in which the receptor binding region is coupled to the hematopoietic growth factor interleukin (IL)-3. This molecule can bind to and activate both the thrombopoietin and IL-3 receptors.

Another growth factor is a thrombopoietin peptide mimetic that consists of a dimer of two identical 14-amino-acid peptides which has no sequence homology with thrombopoietin but avidly binds to and activates the thrombopoietin receptor, c-Mpl.[24] These molecules define a new and growing family of molecules called the Mpl ligand family based upon their common ability to bind and activate the receptor for thrombopoietin, c-Mpl, and are now being studied in clinical trials.

Clinical application of megakaryocyte growth factors

Cancer and leukemia chemotherapy patients use approximately 25% of all platelet products transfused in the United States and are a major area in which thrombopoietin might show benefit in the primary or secondary prophylaxis of thrombocytopenia. A variety of studies demonstrate that thrombopoietin has activity in the chemotherapy setting and is safe, as no adverse events were attributed to the PEG-rHuMGDF. In some series using a dose-intense chemotherapy regimen to treat patients with ovarian cancer, recombinant human thrombopoietin elevated nadir platelet counts, reduced the duration of thrombocytopenia, and produced a 50% reduction in platelet transfusions.[25–27]

In contrast to chemotherapy for solid tumors, the administration of thrombopoietin following chemotherapy for acute leukemia has failed to produce enhancement of platelet recovery when given after standard induction regimens. The reasons for this failure are not entirely clear. They may relate to the absence of target marrow progenitors upon which to act, high endogenous thrombopoietin levels, or an inappropriate administration scheme. Attempts to increase the PEG-rHuMGDF dose and alter the dosing scheme have not met with success.[28]

The thrombocytopenia in cirrhosis may be responsive to treatment with these agents. Although it has long been felt that the thrombocytopenia in liver disease was due to sequestration of platelets in the spleen, most thrombopoietin is produced in the liver, suggesting that insufficient thrombopoietin production may be an additional cause. In one series, 39 of 44 patients with cirrhosis and thrombocytopenia had undetectable serum thrombopoietin levels that increased in 16 of 17 after orthotopic liver transplantation. Serum thrombopoietin concentrations increased significantly on the first day after transplantation, preceding the rise in the peripheral platelet count by 5 days.[29,30]

Risks of thrombopoietin

For thrombopoietin a number of actual or potential toxicities have been identified. Formation of other antibodies to recombinant human hematopoietic growth factors has been uncommon but this has become a significant problem in the clinical development of thrombopoietin. In one report, approximately 4–8% of normal volunteers paradoxically developed thrombocytopenia after receiving three monthly injections of PEG-rHuMGDF; this was apparently due to the development of antibodies to PEG-rHuMGDF (a truncated, non-glycosylated, and pegylated derivative of rHuTPO) that cross-reacted with endogenous thrombopoietin, neutralized its biologic activity, and produced thrombocytopenia. Platelet counts as low as 4000/μL were reported. All patients eventually recovered once the antibody abated but clinical development of this molecule by the manufacturer was stopped in 1998 because of this side effect. In comparison, administration of the parent molecule recombinant human thrombopoietin has not been associated with neutralizing antibodies and remains under development.[31]

None of the closely followed animal or human studies with the thrombopoietins has shown evidence for increased thrombotic events. However, there are three potentially prothrombotic attributes of the thrombopoietins that deserve attention. First, these molecules are extremely potent growth factors and can markedly elevate the platelet count in a short period of time. In a baboon model, the deposition of platelets in an extravascular shunt, which mimics an ulcerated atheroma in humans, was directly related to the platelet count after PEG-rHuMGDF administration. Except for its ability to elevate the platelet count, PEG-rHuMGDF did not synergize with or exacerbate platelet deposition. Nevertheless, increasing the platelet count in individuals with active arterial thrombotic disease may exacerbate the cardiovascular disease.[32,33]

When PEG-rHuMGDF or recombinant human thrombopoietin are added directly to platelets, they decrease by approximately 50% the threshold for activation by various agonists (ADP, collagen) in platelet aggregometry experiments. This may not be clinically relevant since other hematopoietic growth factors have the same effect and have not been associated with thrombotic events.[33]

The production of young platelets is stimulated, leading to a peak in the circulation 4–5 days after administration of PEG-rHuMGDF to normal baboons or humans. These younger platelets have a lower threshold for agonists and are more active in platelet aggregation experiments; however, these effects have not resulted in increased thrombosis in either animals or humans.

Other thrombopoietic growth factors

The interleukins IL-3, IL-6 and IL-11 stimulate platelet production. IL-3 and IL-6 are probably toxic for most clinical uses, but recombinant IL-11 has modest side effects and has been approved by the Food and Drug Administration for use in the prevention of chemotherapy-induced thrombocytopenia. IL-11 stimulates megakaryocyte growth *in vitro* and increases platelet production *in vivo* with a time course similar to that of thrombopoietin. Its thrombopoietic action is not mediated through thrombopoietin release or synergism and is independent of the thrombopoietin receptor. In clinical studies, IL-11 reduces the extent of chemotherapy-induced thrombocytopenia and, in one report, reduced the need for platelet transfusions by 27%. Its major side effects are dilutional anemia, peripheral edema, pleural effusions, and atrial arrhythmias. Papilledema has been described in four of 16 children receiving high doses of this agent (100 μg/kg per day).[34–36]

PLATELET SUBSTITUTES

In the development of oxygen carriers, historically one can recall the use of the term 'blood substitute' or 'artificial blood'. As the field progressed we realized that

these terms were misnomers for what was being developed, namely oxygen carriers. With this realization, one should readily understand how naïve the term 'platelet substitute' is. In considering platelet biology, five major platelet processes occur. These include adhesion, aggregation, secretion, procoagulant activity, and clot retraction. Trying to construct a platelet substitute would be a Herculean task if one were attempting to do all of these. Rather, if some platelet surrogate were to be developed it is likely that one would need to consider an approach that is directed toward accomplishing one of these platelet functions. In fact this has been the general strategy. In one of the earliest approach, augmenting intraplatelet aggregation was achieved by coating red cells with either fibrinogen or fibrinogen peptides.[36] This approach actually permitted correction of the bleeding time in experimental animals. However, no clinical studies were ever attempted.

It has been recognized for some time that the surface of the platelet provides a means for enhancing the activity of prothrombinase complex. Creating a surrogate for the platelet phospholipid function to promote prothrombinase activity is relatively straightforward compared with trying to construct a platelet surrogate that has the important platelet glycoproteins or secretes hemostatically active messenger molecules.

Consequently the approaches that use phospholipid vesicles represent probably the most feasible approach to develop a platelet substitute. While there have been encouraging preclinical studies, clinical trials are yet to be started. Major issues to consider are the prevention of pathologic thrombosis, immune reactions to the substances infused as well as producing a product that has a sustained effect.[37–39]

CONCLUSION

Treatment of bleeding caused by thrombocytopenia or platelet dysfunction may be attempted by use of a variety of pharmacologic agents that augment or stimulate platelet function. In general, these approaches are only effective for a short period of time. A long-term goal is to develop a platelet substitute that provides adequate hemostasis so that patients may be treated over several days. With the development of new platelet growth factors, greater reliance upon stimulating thrombopoiesis may contribute to preventing platelet transfusions in patients with bone marrow failure or chemotherapy.

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